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Are all calories the same? Individual differences in the valuation of protein, carbohydrate and fat

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Abstract

Humans have the ability to identify the macronutrient composition of foods using sensory signals and learned, postingestive feedback. This enables humans to regulate their macronutrient intake and based on physiological needs, protein, carbohydrate and fat are prioritised. The amount of energy metabolised from each macronutrient is different and protein, carbohydrate and fat vary in their utilisation and absorption. However, it is unclear whether protein, carbohydrate and fat are valued equally and whether individual differences influence macronutrient valuations.

The first studies in this thesis developed a novel binary forced-choice task to measure the relative value that individuals place on protein, carbohydrate and fat, calorie for calorie. The binary forced-choice task was an effective measure of macronutrient valuations and there was excellent test-retest reliability. Protein, carbohydrate and fat were not valued equally and there was considerable variation in value across individuals. An additional food category was then added to investigate whether individual's macronutrient valuations are similar for foods consumed at breakfast time and lunchtime. Macronutrient valuations were not consistent across the two meal times suggesting that protein, carbohydrate and fat are valued differently during the day. However, there were concerns with the familiarity of the breakfast food stimuli in this study. The final study investigated whether perceived social status influences the amount of value an individual places on a calorie of protein, carbohydrate and fat. People with lower perceived social status valued protein, carbohydrate and fat to a great extent than people with higher perceived social status.

The experiments in this thesis provide a novel perspective on the ability to identify the macronutrient composition of foods and introduce the idea of macronutrient valuation. Together, the results provide evidence that macronutrients are not valued equally and that the value placed on protein, carbohydrate and fat is moderated by individual differences.

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Author Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

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List of abbreviations

ANOVA - Analysis of Variance

BMI - Body Mass Index (kg/m2)

DASS - Depression, Anxiety and Stress Scale

DEBQ - Dutch Eating Behaviour Questionnaire

DRV - Daily Recommended Value

ED - Energy density

GI - Glycaemic Index

IMD – Index of Multiple Deprivation

Kcal - Kilocalorie

LFPQ - Leeds Food Preference Questionnaire

MJ – Mega joules

MPC - Macronutrient Preference Checklist

MTPRT - Macronutrient Taste Preference Ranking Task

NDNS - National Diet and Nutrition Survey

PANAS - Positive and Negative Affect Scale

SE - Standard error of the mean

VAS - Visual analogue scale

WHO - World Health Organisation

Chapter 1 General Introduction

Humans use both sensory properties and postingestive feedback to discriminate foods based on their macronutrient composition (Birch, 1999). This ability to differentiate the protein, carbohydrate and fat content of foods enables humans to prioritise and value energy based on physiological demands. For example, high-protein foods are valued more after experiencing a period of protein-depletion (Griffioen-Roose et al., 2012). This suggests that the human body can regulate its macronutrient intake in order to replenish and restore nutrient balance. However, it is uncertain whether energy derived from protein, carbohydrate and fat is valued equally, and whether macronutrient value differs across individuals.

Within this thesis, 'macronutrient valuation' is defined as the relative value that humans place on a calorie of protein, a calorie of carbohydrate and a calorie of fat. The differences in value placed on protein, carbohydrate and fat are quantified using a novel binary forced-choice task. The binary forced-choice task was developed to investigate the extent to which individuals prioritise the protein, carbohydrate and fat content of foods when making food choices.

1.1 What are macronutrients?

Food is an essential energy source for all animals, including humans (Kearney & Geissler, 2011). The energy density (ED) of a food is the "amount of energy (kcal) in a particular weight of food and is typically measured as the number of calories per gram of food (kcal/g)" (Rolls, 2009). The macronutrient content of a food will influence its energy density (Geissler & Powers, 2009). However, not all energy is equal, and the three macronutrients; protein, carbohydrate and fat, are different in their absorption and utilization. The amount of energy utilized from protein and carbohydrate is approximately 4kcal/g whereas fat equates to 9kcal/g (Atwater, 1902; Buchholz & Schoeller, 2004).

1.1.1 Carbohydrates

According to UK dietary reference values (DRV) carbohydrates should contribute towards at least half of our daily energy intake (Department of Health, 1991). Carbohydrates are considered the body's main energy source, as they are easily metabolized as glucose and glucose is readily absorbed in to the blood (Swinburn & Ravussin, 1994). Carbohydrates are categorised based on chemical chain length, and carbohydrate-rich foods are commonly grouped as simple sugars (monosaccharides, disaccharides, oligosaccharides) or starches (polysaccharides; Geissler & Powers, 2009).

The glycaemic index (GI) of a food also helps classify carbohydrates. The GI is a measure of the increase in blood glucose after carbohydrate ingestion, compared to a reference value, for example, white bread (Wolever, Jenkins, Jenkins, & Josse, 1991). High glycaemic foods such as potatoes and white-rice are metabolized quickly and cause a rapid increase in blood glucose. Whereas low GI foods such as pulses and oats cause a gradual rise in glucose because they are metabolized slowly.

1.1.2 Fats

Dietary fat is valuable as an energy source as it provides more energy (kcal/g) than protein or carbohydrate. A major function of dietary fat is energy storage, and these reserves are utilised when food intake is reduced or during an overnight fast (Flatt, Ravussin, Acheson, & Jequier, 1985). Fat is also essential for regulating body temperature and protecting vital organs (Skeaff & Mann, 2012). Fats can be divided in to four groups; monounsaturated, polyunsaturated, saturated and trans fatty acids (Yaqoob, Minihane, & Williams, 2011).

Individuals who consume large quantities of trans fatty acids are at a greater risk of experiencing negative health outcomes (Stender & Dyerberg, 2004).

There are two essential fatty acids, omega-3 and omega-6 that the body cannot synthesize, and these must be obtained from food sources such as oily fish and nuts (Innis, 1991). The World Health Organisation (WHO) (Fact Sheet No.394, 2015) recommends that total dietary fat does not exceed 30% of daily energy intake.

1.1.3 Protein

Although dietary protein provides the body with 4kcal/g, unlike fats and carbohydrates, the main function of protein is not as an energy source. Protein is the second largest component of the human body after water, and proteins play an important role in the maintenance and growth of cells, tissue and muscles (Jackson & Truswell, 2012). The DRV for dietary protein is 10 – 15% of energy, or 0.75g of protein /kg of bodyweight /day (Department of Health, 1991).

Proteins are composed of chains of amino acids and there are 21 amino acids that the human body requires (Millward, 1999). Of those 21 amino acids, there are nine that are considered essential and need to be synthesized from food sources (Elango & Laviano, 2017). Foods that contain all nine essential amino acids are considered 'complete protein' sources, for example fish, poultry and eggs (Hoffman & Falvo, 2004). Some foods, such as vegetables and nuts, are considered 'incomplete protein' sources and contain some of the essential amino acids but not all nine. Therefore, it is important that protein is considered as part of the diet in terms of quantity (absolute amounts) and quality (amino acid profile).

1.1.4 Effect on satiety

Appetite refers to the increase and decrease of an individual's urge to eat, also known as hunger. Hunger serves a biological purpose, and human's perceived feelings of hunger motivate them to search and consume food (Blundell, Lawton, Cotton, & Macdiarmid, 1996). There are two processes involved in regulating hunger and food consumption, known as satiation and satiety. Satiation and satiety are often used interchangeably but there is an important distinction. Satiation reduces feelings of hunger and usually brings the eating period to an end. Satiety develops after satiation and delays the onset of hunger (Blundell & Bellisle, 2013).

When compared as isocaloric, macronutrients have different impacts on satiety. Previous research has investigated the satiety value of protein and the results suggest that calorie for calorie, protein is more satiating that carbohydrate or fat (Halton & Hu, 2004). The type of carbohydrate can influence the effect on satiation and satiety. For example, starch with a high dietary fibre content has a higher satiety value than simple sugars (Howarth, Saltzman, & Roberts, 2001). However, all types of carbohydrates exert a greater value of satiation that dietary fat (Green, Burley, & Blundell, 1994). Therefore, a satiation hierarchy is often demonstrated in research; protein > carbohydrate > fat.

High-fat foods are more energy dense than protein and carbohydrate containing foods. Energy dense foods are usually rated as more palatable, but less satiating, and therefore it is easy for people to over consume high-fat, palatable foods (Drewnowski, 1997). In order to maintain a healthy weight, foods with a high-protein content and low energy density should be consumed regularly. These types of foods have a higher satiety value, and therefore prolong feelings of hunger and reduce overall energy intake.

1.2 Macronutrient regulation

As previously mentioned, carbohydrate, protein and fat each have specific roles within the human body, and therefore maintaining and regulating macronutrient balance is critical for survival. In the UK, fat and carbohydrate intake as a percentage of energy have remained fairly constant over the last decade, whereas protein intake has gradually increased (Whitton et al., 2011).

1.2.1 *Oxidation and storage*

Once digested, macronutrients are oxidized for use as energy or stored as energy reserves. The oxidation of one nutrient will tend to suppress the oxidation of another, and there is a hierarchy in which nutrient oxidation occurs (Jebb & Prentice, 2001). As the preferred energy source, it is essential that the central nervous system receives a continuous supply of glucose (Swinburn & Ravussin, 1994)**.** Therefore, carbohydrate oxidation takes priority over protein and fat oxidation. An increase in carbohydrate intake will subsequently increase carbohydrate oxidation (Flatt, 1987). This association is similar for protein intake; the addition of protein to a meal will stimulate protein oxidation (Frayn, 2010). However, fat intake does not influence fat oxidation, but rather it is influenced by the intake of protein and carbohydrate (Stubbs & Elia, 2001).

Despite the importance of glucose as an energy source, there are limited carbohydrate stores which are therefore tightly regulated (Acheson et al., 1988). Protein serves as a structural nutrient, aiding in muscle maintenance and growth, and it is not primarily utilised by the body as an energy source. Therefore protein stores are also limited and tightly regulated (Simpson & Raubenheimer, 2005). The human body's ability to store fat is virtually unlimited and fat stores serve as an adaptive buffer for starvation (Galgani & Ravussin, 2008). Fat intake is not tightly regulated, and any excess energy is converted to fat stores as energy reserves.

1.2.2 *Protein Leverage Hypothesis*

Protein intake is tightly regulated, and according to FAOSTAT (2002) in the US, in terms of absolute amounts consumed and as a percentage of energy, protein intake has remained far more constant over time, more so than fat or carbohydrate intake. The protein leverage hypothesis (Simpson & Raubenheimer, 2005) suggests that protein intake is prioritized over carbohydrate and fat intake, in relation to regulating food consumption. Humans are driven to meet a protein 'target' that provides a sufficient intake of essential amino acids. In the modern feeding environment, foods that have a high- carbohydrate and high- fat content are cheaper and more readily available, than protein containing foods (Brooks, Simpson, & Raubenheimer, 2010). This can therefore lead to an unbalanced diet, resulting in the overconsumption of high- carbohydrate and high- fat foods, in a bid to meet a protein 'target'. In a study by Gosby et al., (2011) participants were given a diet containing 10%, 15% or 25% of energy as protein. Participants who had consumed the 10% energy as protein diet increased their overall calorie intake, compared to participants who had consumed the 15% and 25% energy as protein diet. However, the increase in overall energy intake did not sufficiently replenish protein intake. Foods with a high- carbohydrate and fat content are less satiating that foods with a high- protein content, and therefore participants could have increased their overall energy intake in an attempt to reach the same level of satiation as they would on a protein-rich diet. This suggests that the protein-leverage hypothesis is incomplete, and further research needs to be conducted in order to understand if people are increasing their food intake to reach a 'protein target'.

1.2.3 Nutrient - seeking behaviour

If animals, including humans, do have the ability to regulate macronutrient intake, then nutrient-seeking behaviour should be observed when access to a specific macronutrient is restricted (DiBattista, 1991). After receiving a nutrient-deficient diet, it is expected that the animal will increase their consumption of the previously restricted macronutrient when it becomes accessible in order to restore nutrient balance.

1.2.3.1 Protein

Evidence suggests that the detection of a protein deficiency actively leads to restoration of protein intake. A study using rats found that when in a protein deficit, rats increased their selection and ingestion of protein-rich foods (DiBattista, 1991; Piquard, Schaefer, & Haberey, 1978). Recently, these protein-seeking behavioural mechanisms have been observed in humans. In a study by Griffioen-Roose et al. (2012) participants were given either a high- (21% energy as protein) or low (5% energy as protein) isocaloric diet for 14-days, and were then given access to an ad-libitum feeding phase of 2.5-days. Participants in the low-protein condition ate considerably more high-protein foods, compared to the high-protein condition. Protein intake increased by 13% after the low-protein diet. There was no difference in overall energy intake between the high- and low-protein conditions. This suggests that participants in the low-protein condition actively sought foods that had a higher protein content. Participants might have experienced a protein-specific appetite in order to replenish their depleted protein stores. This supports the idea that humans have the ability to regulate their protein intake. However, it is unclear whether participants selected the foods based on a preference for the protein content, or the savoury flavouring of the foods. The low- and high-protein diets included a similar number of sweet-flavour foods (53 and 54, respectively) but the low-protein diet had fewer savoury-tasting foods than the high-protein diet (9 and 15, respectively).

Participants in the low-protein condition might have experienced 'sensory-specific satiety' and increased their intake of savoury-flavoured foods after consuming a larger quantity of sweet-flavoured foods. As a food is eaten, its taste decreases in pleasantness but the pleasantness of other foods remain relatively unchanged; this is referred to as 'sensory-specific satiety' (Rolls, Rolls, Rowe & Sweeney, 1981). Sensory-specific satiety is thought to encourage a balanced diet with a varied of nutrients (Rolls, Hetherington & Burley, 1988), and helps to explain why after a savoury meal, there is still a desire to consume a sweet desert (Hetherington, 1996). Future studies should include an equal amount of sweet and savoury foods in the low- and high- protein diets, in order to determine whether participants were increasing their protein intake to replenish a deficiency or because the savoury-tasting food appeared more desirable after consuming sweet-tasting foods.

1.2.3.2 Fat

There is evidence to suggest, in animal studies, that behavioural mechanisms exist to regulate fat intake. Rats were fed an omega-3 fatty acid deficient, or replete diet, for four-weeks (Dunlap & Heinrichs, 2009). The omega-3 deficient rats preferred and consumed more of a solution with a high omega-3 fatty acid content compared to the control rats. This suggests that the rats could identify a nutrient deficiency, and select foods based on their nutrient content in order to replenish their omega-3 fatty acid intake.

There is currently no evidence to suggest that humans have the ability to regulate their fat intake through nutrient–seeking behaviour. A possible explanation for this is that the human body has a large capacity for fat reserves (Flatt et al., 1985), and therefore experiencing a fat-deficiency is unlikely. Although omega-6 and omega-3 fatty acids are essential nutrients (Simopoulos, 1999), the mechanism used to regulate intake may not be sensitive enough to identify a specific dietary fat deficiency.

1.2.3.3 Carbohydrate

The evidence for carbohydrate-seeking behaviour in animal and human studies is mixed. Wurtman, Moses, and Wurtman (1983) found evidence to support carbohydrate-seeking behaviour in rats. After a carbohydrate-restricted diet, rats significantly increased their intake of carbohydrate-rich foods compared to a control group that had access to carbohydrates. Whereas, when DiBattista (1991) fed rats either a carbohydrate-deficient diet or a control diet (access to protein, carbohydrate and fat), the carbohydrate-restricted rats did not increase their intake of the carbohydrate solution relative to the control group.

There is some evidence for carbohydrate-seeking behaviour in human studies. Participants who restricted their carbohydrate intake reported increased cravings for, and increased intake of, carbohydrate-rich foods in comparison to a control group (Coelho, Polivy, & Herman, 2006). Although, there was a recruitment concern within this study as more "carbohydrate-cravers" were assigned to the carbohydrate-restricted condition than the control condition. Therefore, it is difficult to determine whether carbohydrate intake increased because of the restricted diet or because the participants were characterised as having an overwhelming desire to consumed carbohydrate-rich foods (carbohydrate-cravers; Wurtman & Wurtman, 1995).

1.3 Macronutrient identification

In order to regulate macronutrient intake, mechanisms must exist that enable humans to discriminate the protein, carbohydrate and fat content of a food. Both innate taste preferences, and learned experiences help detect necessary nutrients. The visual appraisal, texture, taste and smell of a food can influence food choice and energy intake (Mccrickerd & Forde, 2016). After repeated exposure and learning, the sensory properties of food can signal the postingestive consequences of eating that food. As a result of this learning, expectations about foods develop, and these can drive food choice.

1.3.1 Taste as a nutrient-sensor

Taste is an important sensory signal for the nutritional content of a food and there are five primary tastes; salt, sweet, umami, sour and bitter (Boesveldt & de Graaf, 2017). From birth, humans have innate preferences for specific tastes. When infants are given a sweet flavour solution, they elicit pleasure behaviours such as smiling (Ventura & Worobey, 2013). Sweet tastes are associated with energy and therefore this preference may have developed as an adaptive behaviour to seek out energy dense foods (Drewnowski, 1995). Bitter tastes are commonly disliked by infants and elicit avoidant behaviour (Birch & Fisher, 1998). This may also be an adaptive response as bitter tastes can be associated with toxic foods.

The macronutrient content of foods can be identified with taste associations. Sweet tastes are associated with carbohydrate containing foods; especially those with a high sugar content, and savoury tastes are associated with protein containing foods (van Langeveld et al., 2017). The taste associated with fat content is less clear because none of the primary taste categories signal the fat content of a food (Liu, Archer, Duesing, Hannan, & Keast, 2016).

Learned taste associations enable humans to regulate nutrient intake, although in some cases the taste of a food is not synonymous with its nutritional content. The ability to identify the nutrient content of highly processed foods based on taste can be difficult (Van Dongen, Van Den Berg, Vink, Kok, & De Graaf, 2012). Often, highly processed foods have been developed to increase palatability, reduce energy density and to extend food "shelf-life". This involves additional artificial flavourings, sweeteners and additives that can alter the original taste of a food. This can result in inconsistent taste and nutrient relationships. For example, crisps are a savoury-flavoured snack food that has a low-protein content. Eating raw and moderately processed foods is the best way to ensure that the nutrient content of a food is successfully predicted.

1.4 Macronutrient intake and individual differences

There is considerable variation in individual's dietary decisions that ultimately, influences macronutrient intake. There are many factors that influence an individual's food choice, but for the purpose of this thesis, environmental (socioeconomic status), biological (age and health) and subjective (mood) factors are discussed.

1.4.1 Age

Macronutrient requirements change over the human lifespan from infancy through to the elderly (Langley-Evans, 2015). Appetite and food intake reduce with age, and this increases the risk of energy imbalance and inadequate nutrient intake (Morley, 2001). Ageing is also associated with reduced muscle mass and therefore maintaining a balanced intake of protein is essential**.** Therefore, it is important that the elderly population adjust their macronutrient intake in order to avoid negative health outcomes e.g. Sarcopenia – the loss of muscle mass (Beasley, Shikany, & Thomson, 2013). For a healthy adult, the recommended intake of protein is 0.75g /kg of bodyweight /day, in comparison, the recommended protein intake for the elderly population is approximately 1.5g /kg of bodyweight /day (Wolfe, Miller, & Miller, 2008). This highlights the importance of adjusting dietary macronutrient intake over the lifespan.

1.4.2 Socioeconomic status and perceived social status

The relationship between obesity and socioeconomic status has been extensively researched and results suggest that in high-income countries, people with lower socioeconomic status are more likely to be obese compared to people with higher socioeconomic status (Baum & Ruhm, 2009; Nettle, Andrews, & Bateson, 2017). The increased risk of obesity in lower socioeconomic status groups could be related to their energy and macronutrient intake. People with lower socioeconomic status tend to consume less whole grains, lean meats, and fruit and vegetables in comparison to high socioeconomic status groups (Giskes, Turrell, Patterson, & Newman, 2002; Lang, Thane, Bolton-Smith, & Jebb, 2003). Low socioeconomic status adults consume a larger quantity of high-fat, energy-dense foods that are often cheaper than low-energy foods such as fruits and vegetables (Darmon, Briend, & Drewnowski, 2004). It is cheaper to buy energy-dense foods because as the energy density (MJ/kg) of a food increased the energy cost (£/MJ) decreases

(Drewnowski & Specter, 2004). There is also a discrepancy in the cost of protein, carbohydrate and fat (£/MJ) where high-protein foods tend to cost more than high-carbohydrate/low-protein foods (Brooks et al., 2010). High-carbohydrate and high-fat foods are the best energetic value for money and are therefore consumed in larger quantities than high-protein foods by those who are financially insecure.

The relationship between lower socioeconomic status and obesity is mediated by a multitude of factors, and there are people with lower socioeconomic status that maintain a healthy weight. Recently, researchers have been focusing on the influence of perceived social status and obesity. Perceived social status is defined as an individual's subjective social standing in their community, relative to others (Adler, Epel, Castellazzo, & Ickovics, 2000). Evidence suggests that feelings of low perceived social standing can increase food intake and subsequently obesity (Goodman et al., 2003). This association may have served as an adaptive advantage because as social animals, human survival depends on both food sources and non-food sources (Cheon, Lim, McCrickerd, Zaihan, & Forde, 2018).

1.4.3 Mood

There is evidence suggesting that some people choose specific foods in response to their emotional state. Macht (2008) conducted a survey and found that on average 30% of people increase their food intake when experiencing feelings of stress. Stressful situations can trigger stress-induced eating in an attempt to reduce feelings of negative affect (Gibson, 2012). For example, during a stressful exam period, students increased their carbohydrate and saturated fat intake, compared to the beginning of term (Roberts, Campbell, & Troop, 2014). Also, during stressful work periods, workers reported eating less vegetables and more high-fat, sweet foods (O'Connor, Jones, Conner, McMillan, & Ferguson, 2008).

Some people, often referred to as 'carbohydrate-cravers', choose to increase their carbohydrate intake in an attempt to self-medicate and reduce feelings of depression

(Corsica & Spring, 2008; Wurtman & Wurtman, 1995). de Castro (1987) found that an increase in energy consumption from carbohydrates decreased symptoms of depression. Also, chocolate (a high-carbohydrate food) is reported as the most commonly consumed food when experiencing negative affect or stress (Gibson, 2006). Similarly, when participants received low-carbohydrate, high-protein meals over one week, they showed an increase in behaviour related to anger, depression and tension (Keith, O'Keeffe, Blessing, & Wilson, 1991).

These results suggest that an individual's mood can influence macronutrient intake. Previous research has demonstrated that high-carbohydrate, sweet foods are preferred in situations associated with negative mood (Benton, 2002). The hedonic reward associated with high-carbohydrate, sweet foods might explain why negative feelings are reduced after consumption. These types of foods are often considered palatable and therefore pleasurable to eat and digest (Berridge, 1996). However, not all individuals increase their food intake in response to negative emotions. As demonstrated in the survey conducted by Macht (2008), an average of 48% of people report eating less and experiencing reduced appetite.

1.5 Key findings in the literature

From this review of the literature, there is some evidence to suggest that humans have the ability to regulate their macronutrient intake using behavioural mechanisms. Despite these regulatory mechanisms, macronutrient intake differs across individuals. A possible explanation for these differences involves the underlying value that is placed on protein, carbohydrate and fat, on a calorie for calorie basis.

1.5.1 What is macronutrient value?

Macronutrient valuation is a novel concept, introduced in this thesis to investigate whether the macronutrient content of a food contributes towards an individual's food choices.

“Macronutrient valuation” refers to an individual’s underlying disposition to select foods according to how macronutrients are prioritised. For example, two foods might have a small difference in protein content and the same carbohydrate and fat content (kcal/g). If the food with the higher protein content is selected, then this is evidence of protein valuation. Protein valuation may be higher after a period of protein depletion. In order to replenish protein stores, protein is then prioritised and therefore valued in food choices. Research identifying differences in macronutrient valuation across individuals could provide insight in to food choice motivations and could help guide specific dietary interventions. Macronutrients differ in their metabolisable energy, functions, oxidation and their effect on satiety. Therefore, it is predicted that macronutrients will be valued differently, both within and between individuals.

Liking and food preferences are other measures used to determine food choice motivations. These subjective measures are often influenced by our environment and can be subject to change. For example, babies usually demonstrate dislike for bitter tastes such as caffeine but this dislike can change with age and many adults record enjoying the taste of coffee (Birch, 1999). It is predicted that individual’s macronutrient valuations will remain consistent from one time period to another; this is explored with a test-rest measure. However, it is less clear whether macronutrient valuations will remain stable across different foods and different meal times.

1.6 Aims of the thesis

The aims of this thesis are to investigate the relative value placed on protein, carbohydrate, and fat, on a calorie for calorie basis, and whether the value is influenced by individual differences, specifically perceived social status. In order to compare each macronutrient relative to the other, protein, carbohydrate and fat are compared calorie for calorie instead of as their absolute energy value. Chapter 2 is split in to part 1 and part 2 and discusses the development of a novel task to investigate macronutrient valuation. Part 1 explores the feasibility of using a binary forced-choice task to measure macronutrient valuation, and part 2 measures the test-retest of individual's protein, carbohydrate and fat valuations across two-test sessions. Chapter 3 investigates individual's macronutrient valuation in lunchtime foods and breakfast cereals, and whether these valuations remain constant across the two different mealtimes. Chapter 4 uses the binary forced-choice task to investigate the influence of perceived social status on macronutrient valuation, independent of socioeconomic status.

The order of this thesis is logical rather than chronological. The same data set is used in chapter 2, part 2, and chapter 4. The participants, methods and results are identical. The statistical analysis and the results are different in Chapter 2, part 2, and Chapter 4 and are relevant to the questions being investigated. The data was collected to investigate two separate research questions. The first question investigated the test-retest reliability of the binary forced-choice task to measure macronutrient valuation and therefore it is included in Chapter 2 as it contributes towards the task development. The second question investigated the effect of perceived social status on macronutrient valuation and is discussed in Chapter 4.

Chapter 2 Development of a novel, binary forced-choice task to measure macronutrient valuation

2.1 Introduction

The aim of the following studies was to develop and test a novel, binary forced-choice task to investigate macronutrient valuation. "Macronutrient valuation" is defined as the relative value that humans place on a calorie of protein, a calorie of carbohydrate and a calorie of fat. It is important to reiterate that, in this thesis, macronutrient value is measured on a calorie for calorie basis. This is to ensure that the three macronutrients are compared relative to one another.

As discussed in Chapter 1, macronutrient intake is regulated, and humans have the ability to differentiate the macronutrient composition of food. Despite macronutrient intake remaining relatively constant in the UK, over the last decade (Whitton et al., 2011), individuals differ in their energy intake and body mass index (BMI; Kelly, Patalay, Montgomery, & Sacker, 2016). Some individuals might be more sensitive than others to the macronutrient composition of food, making it easier for them to identify and regulate their macronutrient intake. Therefore, using a forced-choice task to quantify the value that humans place on protein, carbohydrate and fat could give an insight in to humans' ability to identify the macronutrient content of foods. However, at the moment, there are currently no tasks that specifically measure macronutrient valuation.

Previous research has focused on the influences that macronutrient preferences have on food choice and energy intake (Drewnowski & Hann, 1999). Research measuring macronutrient preferences has generally found that foods that have a high-fat and high-sugar content are preferred more than foods with a low-energy content (Drewnowski, 1995; Johnson, McPhee, & Birch, 1991). However, the methodology varies, for example, The Macronutrient Preference Checklist (MPC; Hill, Leathwood, & Blundell, 1987) is a 32-item checklist used to measure momentary macronutrient and taste preferences. The MPC included four food groups; high-carbohydrate, high-fat, high-protein and low-energy. Participants were instructed to "check off all food items that they felt like eating at that moment and to consider each item independent of one another". Participants also rated their liking of the 32 food items using a 9-point hedonic scale.

The MPC is a convenient and easy-to-complete tool for measuring macronutrient preferences, and the food can be changed to represent commonly consumed foods in the country of interest, for example the USA (Brisbois-Clarkson, McIsaac, Goonewardene, & Wismer, 2009). However, a limitation of the MPC is that food items are listed as words rather than as images, and this requires participants to mentally picture the food items. It is important to use images because visual cues can signal expected satiety and palatability, both key factors that influence food choice (Mccrickerd & Forde, 2016).

The Macronutrient Taste and Preference Ranking task (MTPRT; de Bruijn, de Vries, de Graaf, Boesveldt, & Jager, 2017) is another method developed to investigate macronutrient preferences and food choice. Similarly, to the MPC, the MTPRT included 32 food items, eight foods in each of the four categories: high-fat, high-carbohydrate, high-protein and low- energy. The MTPRT used images of foods instead of words. During the task, participants were shown four food images and asked to rank the images in order of "what they most desire to eat at this moment". Participants first clicked on the most preferred food image, followed by their second and third preferred foods and finally the least preferred food. An overall macronutrient preference score was calculated by adding up the individual preference scores for each food, in each category. The ranking aspect of the MTPRT creates an order of macronutrient preference.

An alternative method used to measure food preferences is the forced-choice paradigm. The Leeds Food Preference Questionnaire (LFPQ; Finlayson, King, & Blundell, 2007) uses the forced-choice methodology to measure participants 'wanting' of high- and low- fat foods. Brunstrom, Drake, Forde and Rogers (2018) also used a forced-choice task to quantify the value that participants placed on foods with varying energy densities. In a forced-choice design, participants are presented with two stimuli simultaneously and asked to make a choice between these stimuli, based on a previously given criterion. A key aspect of the forced-choice design is that participants must make a choice between the two stimuli and they are not given the option to avoid the decision or skip a trial.

The current research comprised two studies that use different data sets and methodologies. Both studies contribute towards developing a novel binary forced-choice task to measure participant's relative value of a calorie of protein, a calorie of carbohydrate and a calorie of fat.

Part 1 investigated the feasibility of the binary forced-choice design in quantifying humans' macronutrient valuation. The aim was to determine whether protein, carbohydrate and fat are valued equally, calorie for calorie. Two different food lists were included to investigate whether participants' macronutrient valuations were the same across two different food lists, in two separate sessions. Based on previous research reviewed in Chapter 1, it was predicted that the macronutrients would be valued differently; specifically protein will be valued the most, followed by carbohydrate, and that fat will be valued the least.

Part 2 aimed to assess the test-retest reliability of the binary forced-choice task as a measure of macronutrient valuation, within one food list, across two test sessions. The key prediction for Part 2 was that participants' relative value for protein, carbohydrate and fat, on a calorie for calorie basis, would remain constant across two test sessions, one-week apart.

2.2 Part 1: Feasibility of a binary forced-choice task to measure macronutrient valuation

2.2.1 Methodology

The aim for this study was to identify whether the novel binary forced-choice task could be used to measure and quantify the underlying value that individuals place on protein, carbohydrate and fat, calorie for calorie. This pilot study involved participants choosing between several foods, in forced-choice trials, in order to determine whether the macronutrient composition determine their food choice. Participants expected satiety and familiarity of the food was also measured.

2.2.1.1 Design

The experiment used a repeated-measures design. The independent variables were the carbohydrate, protein and fat content of the test foods and the dependent variable was food choice.

2.2.1.2 Participants

Seven female and seven male participants aged 21 - 51 years (*M*= 28.21, *SD*= 9.62) were recruited to the study and were all colleagues of the experimental team. One (female) participant did not complete both testing sessions and is not included in the analysis. The remaining 13 participants attended both session 1 and session 2 and each participant completed the experiment in the psychology department cafe. The exclusion criteria for this study included vegetarians, vegans, food allergies and food intolerances.

2.2.1.3 Food stimuli

The first test session included images of 21 foods and the second test session included images of 20 foods (see *Table 2-1* and *Table 2-2* for a full list of the nutritional information). The foods selected were based on commonly consumed foods, according to the National Diet and Nutrition Survey (NDNS; Henderson, Gregory, & Swan, 2002) and based on their macronutrient composition. It was important to include a range of high-protein, high-carbohydrate and high-fat foods. The correlations between macronutrients in session 1; protein and carbohydrate ($r= -0.341$, $p= .141$), fat and carbohydrate ($r= 0.120$, $p= .615$) and protein and fat ($r= 0.127$, $p= .593$) and session 2; protein and carbohydrate ($r= -0.482$, $p< .032$), fat and carbohydrate ($r= -0.156$, $p= .512$) and protein and fat ($r= 0.328$, $p= .159$) were weak.

The food was photographed using a high-resolution camera that was positioned at a 45-degree angle. Each food item was photographed in 100-g portions, in the centre of a white plate (round plate 225-mm diameter). The name of the food was presented in the top-left-hand corner of the image, to help participants identify the food. All food photographed was purchased from J. Sainsbury PLC and nutritional information was obtained from food packaging and the Sainsburys website.

2.2.1.4 Measures

Food Choice:

Participants completed a binary forced-choice task (Brunstrom et al., 2018) that instructed them to “Imagine that you are only allowed to eat once, between breakfast and dinner at 7pm. Only these portions are available, and you can only choose one!” Two images of food were then presented side by side and participants chose the left or right food image by pressing the left or right arrow on the keyboard. All the food items were compared to one another, resulting in 210 trials for session 1 and 190 trials for session 2. The trials were randomly generated to be different for each participant, and it was not possible to skip a trial. Figure 2-1 displays an example of one of the binary forced-choice trials.

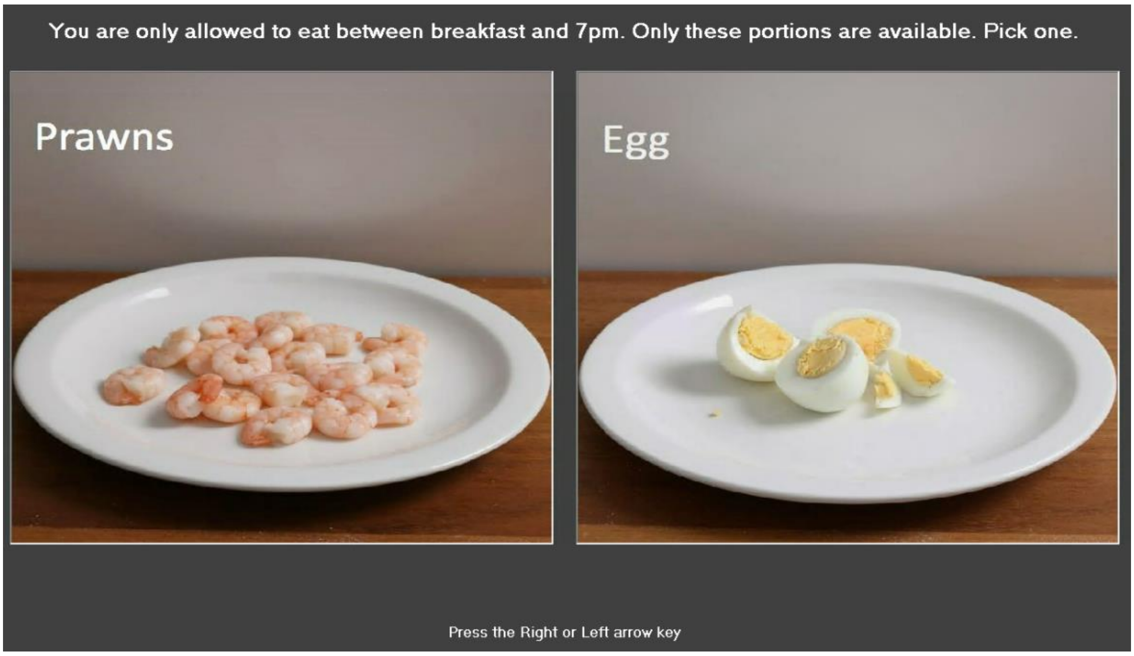

Figure 2-1 Example of a binary forced-choice trial

<u>Expected Satiety:</u>

As in a study by, Brunstrom and Rogers (2009), participants were presented with one of the test foods on the left of the screen and a portion of "Uncle Ben's classic pilau rice" on the right of the screen. Following instructions, participants looked at both food images and changed the portion of rice on the right so that it would prevent hunger for the same amount of time as the portion of food on the left. The plate of rice could be changed in 20-kcal increments using the left and right arrow key. The rice portion ranged from 20-kcal to 800-kcal, and the presented portion size was randomly generated for each participant. The rice portion had to be changed by at least one 20-kcal increment before participants could move on. This was to ensure that participants did not always agree with the presented portion. Rice was used as the comparison food as it is a commonly consumed food in the UK

(Whitton et al., 2011). In session 1 and 2, all foods were presented alongside the portion of rice so that every food had an expected satiety score.

Familiarity:

Participants were shown a food image with the question "Have you eaten this food more than 10 times?" To answer, participants clicked on the "Yes" or "No" button. This was repeated for all the foods in the test sessions.

2.2.1.5 Procedure

Participants all completed the study in the psychology department building. Participants completed the session at a convenient time for both the experimenter and the participant. All participants were sat at a desk and completed the measures on a University of Bristol laptop. Before starting, all participants were given an information sheet and gave consent to the experimenter. Participant's gender and age were recorded. The food choice measure was presented first, followed by expected satiety, and finally the familiarity measure. It was important that the food choice measure was first so that participants were seeing the food images for the first time when making their selection. Session 2 was completed two weeks after session 1, and the procedure was identical except for the different food stimuli used. This study received ethical approval from the University of Bristol, Faculty of Science Ethics Committee (44841).

Table 2-1 *Macronutrient composition and energy density of the food in session 1 in g/100g and kcal/100g*

	Nutrient (g) per 100g					Energy (kcal) per 100g			
Food	Fat	Carbohydrate	Protein	Sugar	Salt	Fat	Carbohydrate	Protein	Total
Birds Eye cod fish fingers	9	21	13	0.88	1.1	81	84	52	219
Birds Eye potato waffles	8.7	22	2.5	0.75	0.70	78.3	88	10	180
Green Giant original Sweetcorn	1.7	11.8	2.4	0.4	7.4	15.3	47.2	9.6	77
Hula Hoops original crisps	26	63	3.3	1.4	0.6	234	252	13.2	507
New York Bakery Co. bagel	1.2	50.1	10.1	0.8	5.60	10.8	200.4	40.4	258
Asparagus spears	0.5	0.7	1.6	0.07	0.7	4.5	2.8	6.4	15
Blueberries	0.5	9.1	0.9	9.1	0.01	4.5	36.4	3.6	45
Closed cup white mushrooms	0.5	0.5	1.8	0.5	0.50	4.5	2	7.2	16
King prawns	0.5	0.5	14.1	1.5	0.50	4.5	2	56.4	62
Avocado	19.5	1.9	1.9	0.02	0.50	175.5	7.6	7.6	198

British ham slices	2.9	0.9	18.8	1.63	0.90	26.1	3.6	75.2	105
British mature cheddar cheese	34.9	0.5	25.4	1.8	0.50	314.1	2	101.6	416
Chargrilled chicken slices	1.6	0.5	23.9	0.2	0.00	14.4	2	95.6	113
Deli-Style coleslaw	17	5.4	0.8	0.75	4.50	153	21.6	3.2	181
Dried apricots	0.6	36	4	0.04	36	5.4	144	16	178
Fairtrade bananas	0.5	23	1.2	0.01	20.90	4.5	92	4.8	103
Four - bean salad	2.8	11.5	6.6	0.25	1	25.2	46	26.4	107
Free range large eggs	9.6	0.5	14.1	0.38	0.50	86.4	2	56.4	143
Scottish mild smoked salmon	10.3	3.3	20.1	2.8	0.00	92.7	13.2	80.4	188
Tuna chunks in spring water	0.5	0.5	27	0.75	0.50	4.5	2	108	113

Note: All food purchased was Sainsbury's own brand unless stated otherwise in the table

Table 2-2 *Macronutrient composition and energy density of the food in session 2 in g/100g and kcal/100g*

	Nutrient (g) per 100g					Energy (kcal) per 100g			
Food	Fat	Carbohydrate	Protein	Sugar	Salt	Fat	Carbohydrate	Protein	Total
Bernard Matthews turkey breast slices	1.4	2.1	18.6	1.8	0.7	12.6	8.4	74.4	97
Birds Eye chicken nuggets	12	21	15	0.85	0.5	108	84	60	257
Heinz baked beans	0.2	12.9	4.7	0.6	5.00	1.8	51.6	18.8	79
21-day matured beef escalope’s, extra lean	3.6	0.5	36.4	0.15	0.5	32.4	2	145.6	178
Beef meatballs	8	4.1	18.9	0.75	1.7	72	16.4	75.6	164
British smoked mackerel	22.2	0.5	20.8	1.98	0.5	199.8	2	83.2	284
Broccoli	0.6	3.1	4.3	0.02	1.80	5.4	12.4	17.2	40
Butcher's Choice Lincolnshire pork sausages	17	8.4	14	1.53	1.30	153	33.6	56	246
Cod fillets	1.3	0.5	21.5	0.19	0	11.7	2	86	98
Crumpets	1.1	42.6	6.6	1.12	3.90	9.9	170.4	26.4	212

Hash brown	9.3	22.2	2.4	0.49	0.7	83.7	88.8	9.6	189
Mediterranean vegetables	1.7	5.9	1.1	0.03	5.20	15.3	23.6	4.4	47
Mini pork pies	21.4	27	11.6	1.05	1.4	192.6	108	46.4	350
Mixed pulses	0.7	13.7	6.8	0.04	0.7	6.3	54.8	27.2	99
Penne rigate	0.7	32.5	5.1	0.02	1.50	6.3	130	20.4	160
Pink lady apples	0.5	11.8	0.5	0.5	11.80	4.5	47.2	2	47
Potato salad	13.1	10.4	1.8	0.7	2.80	117.9	41.6	7.2	169
Red seedless grapes	0.5	17	0.6	0.01	17.00	4.5	68	2.4	75
Somerset brie cheese	24	0.5	18.3	1.26	0.5	216	2	73.2	291
Sweet potato	0.5	18.9	1.1	0.09	8	4.5	75.6	4.4	87

Note: All food purchased was Sainsbury's own brand unless stated otherwise in the table

2.2.1.6 Statistical Analysis

All data analyses were performed using R software (R Core Team, 2017) and the significance value was set at $p < .05$. The binary logistic regression models were completed using the lme4 add-on package (Bates et al, 2015) and figures were created using the ggplot2 add-on package (Ginestet, 2011). The statistical analysis for session 1 and session 2 were identical. Participants' familiarity with the food stimuli was checked by calculating the percentage of participants that said 'Yes' to the question 'Have you eaten this food more than 10 times?'

Before analysis, the protein, carbohydrate and fat content g/ per 100-g was multiplied by 4, 4, and 9, respectively to calculate the protein, carbohydrate and fat content in kcal/ per 100-g. For each food choice trial, a separate 'difference score' was calculated (right-hand food – left-hand food) for protein, carbohydrate and fat. The 'difference scores' represented the difference in energy content (kcal) from each macronutrient between the two foods displayed on any given trial. A positive difference score means that the right food has more energy for each nutrient, and a negative difference score means that the left food has more energy.

For each participant, and each session, separate binary logistic regression models were used to determine the relative value of protein, carbohydrate and fat in predicting food choice. The macronutrient difference scores were entered into the models as predictors and the outcome variable was food choice. The averaged β coefficients were used to quantify the relative value placed on protein, carbohydrate and fat, calorie for calorie. A large β coefficient indicated that a specific macronutrient influenced choice across trials. The larger the β coefficient, the more influence it has on predicting food choice and therefore the macronutrient with the largest β coefficient is thought to be valued the most, and the macronutrient with the smallest β coefficient is valued the least. The protein, carbohydrate and fat β coefficients can be numerically ordered to create a 'macronutrient valuation hierarchy'.

One-way analysis of variance (ANOVA) tests were conducted to assess whether there was a difference in the amount of value placed on protein, carbohydrate and fat. Macronutrient β coefficients were used as the outcome variable, and the protein, carbohydrate and fat 'difference scores' were used as predictors. A Tukey test was used to determine whether the relative value placed on protein, carbohydrate and fat were significantly different from one another.

In order to determine whether participant's macronutrient valuations were the same across different foods, the participant's β coefficients for protein, carbohydrate and fat in session 1 were correlated with their β coefficients for protein, carbohydrate and fat in session 2. The Pearson's correlation coefficients indicated the strength of the relationship between macronutrient value in session 1 and session 2. A strong correlation coefficient would suggest that participant's value of protein, carbohydrate and fat remained consistent across two different food lists.

2.2.2 Results

2.2.2.1 Participant's demographics

Table 2-3 summarised participant's characteristics, include age and gender.

Table 2-3 *Participant's demographics*

	M	SD	Range
Age (years)	28	9.9	21 – 51
Gender (%Female)	46%		

2.2.2.2 Familiarity of food stimuli

Table 2-4 shows the percentage of people who identified as having eaten the food stimuli in session 1 and session 2 more than 10 times. The majority of the foods were familiar, although some foods, such as pulses (43%), Brie cheese (64%), apricots (57%) and the bean salad (43%) were less familiar. The mean expected satiety scores for each food in session 1 and session 2 are also displayed in *Table 2-4*. The food expected to be the least satiating was apples (151 kcals), and the most satiating was bagels (375 kcals).

Table 2-4 *Percentage of participants who were familiar with the food stimuli and the mean (M) expected satiety ratings for each food in session 1 and session 2*

Food List 1	Familiarity	Expected satiety	Food List 2	Familiarity	Expected satiety
	(%)	Mean (kcal)		(%)	Mean (kcal)
Apricots	57	202	Apple	100	165
Asparagus	79	175	Baked beans	93	180
Avocado	71	272	Brie	64	306
Bacon	100	263	Broccoli	100	191
Bagel	100	375	Cod fillet	100	222
Bananas	93	243	Crumpets	100	286
Bean salad	43	258	Mackerel	86	242
Blueberries	86	195	Grapes (red)	100	171
Cheddar cheese	100	295	Hash brown	79	268
Chicken	100	252	Meatballs	93	218
Coleslaw	86	175	Chicken nuggets	93	240
Egg	86	206	Pasta	100	271
Fish fingers	100	257	Pork pies	71	262
Ham	100	200	Potato salad	79	205
Hula hoops	93	238	Pulses	43	260
Mushrooms	93	151	Sausages	100	235

Prawns	93	223	Steak	93	271
Smoked salmon	86	234	Sweet potato	93	320
Sweetcorn	86	152	Turkey slices	100	285
Tuna	79	245	Vegetables	93	172
Potato waffle	79	280			

2.2.2.3 Quantifying the value of protein, carbohydrate and fat

Table 2-5 displays the mean β coefficients and standard error for protein, carbohydrate and fat in session 1 and session 2. The β coefficient refers to the odds of choosing the right-hand food when the right –hand food contains 1kcal/ 100g more protein than the left-hand food. These β coefficients quantify macronutrient valuation. The β coefficients for carbohydrate and fat can be interpreted in the same way.

Table 2-5 *Summary of binary logistic regression analysis for variables predicting food choice in session 1 and session 2*

	Session 1		Session 2	
	β	SE	β	SE
Protein	0.0110	0.0022	0.0101	0.0040
Carbohydrate	0.0003	0.0015	0.0052	0.0029
Fat	-0.0015	0.0016	-0.0013	0.0021

Note: β= unstandardized regression weight, SE = standard error of β

Figure 2-2 displays the mean macronutrient β coefficients and standard error bars for session 1 and session 2. In both session 1 and session 2, protein had the largest β coefficient, carbohydrate had the second largest β coefficient and fat had the smallest β coefficient. Therefore, the β coefficients represent a macronutrient valuation hierarchy: protein > carbohydrate > fat.

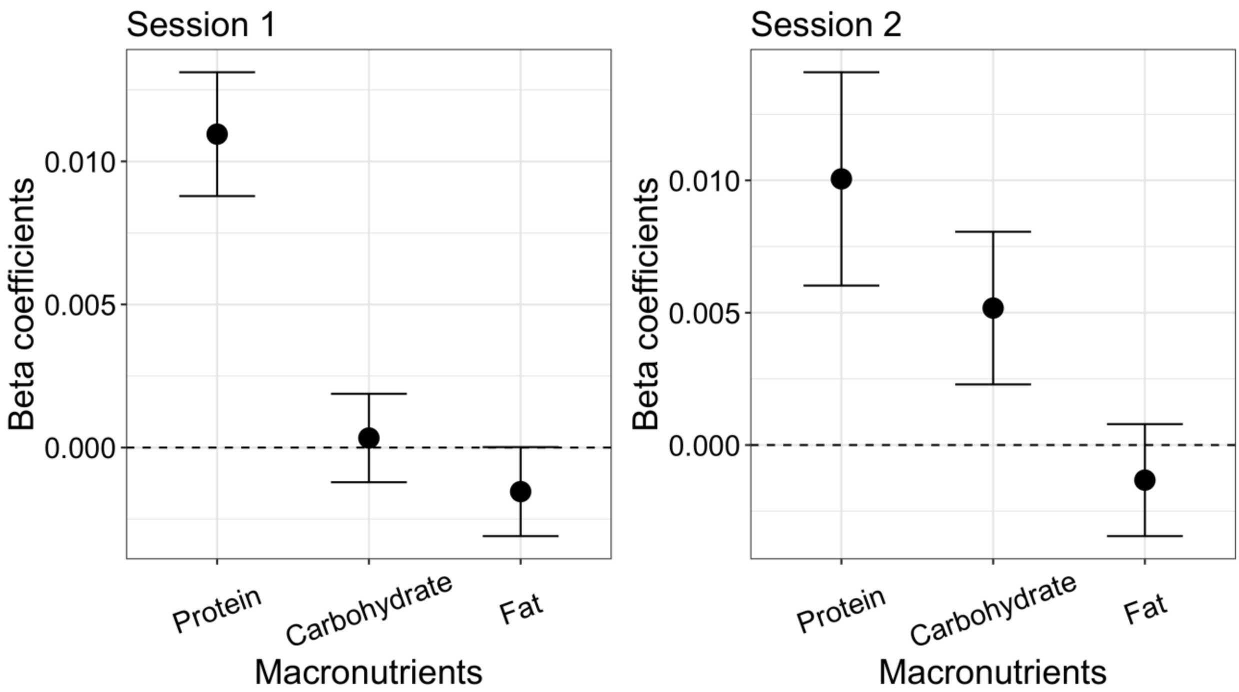

Figure 2-2 *Averaged multilevel binary logistic regression β coefficients and standard error bars for protein, carbohydrate and fat in session 1 and session 2.*

For session 1, there was a significant difference in valuation between macronutrients determined by a one-way ANOVA, $F(2, 36)=14.35$, $p< .001$. A Tukey-adjusted *post-hoc* test specified a significant difference in the valuation of protein compared to carbohydrate ($p< .001$), and in the valuation of protein compared to fat ($p< .001$). There was not a significant difference in the valuation of carbohydrate compared to fat ($p= .739$).

For session 2, the one-way ANOVA indicated that there was a significant difference in the β coefficients for protein, carbohydrate and fat, $F(2, 36)= 3.37$ $p= .046$. A Tukey-adjusted *post-hoc* test indicated that the value placed on protein was significantly greater than the value placed on fat ($p < .001$). There was not a significant difference in the valued placed on carbohydrate compared to fat ($p = .314$) or in the valuation of protein compared to carbohydrate ($p = .515$).

Figure 2-3 shows the relationship between participants' β coefficients for protein, carbohydrate and fat in session 1 and session 2.

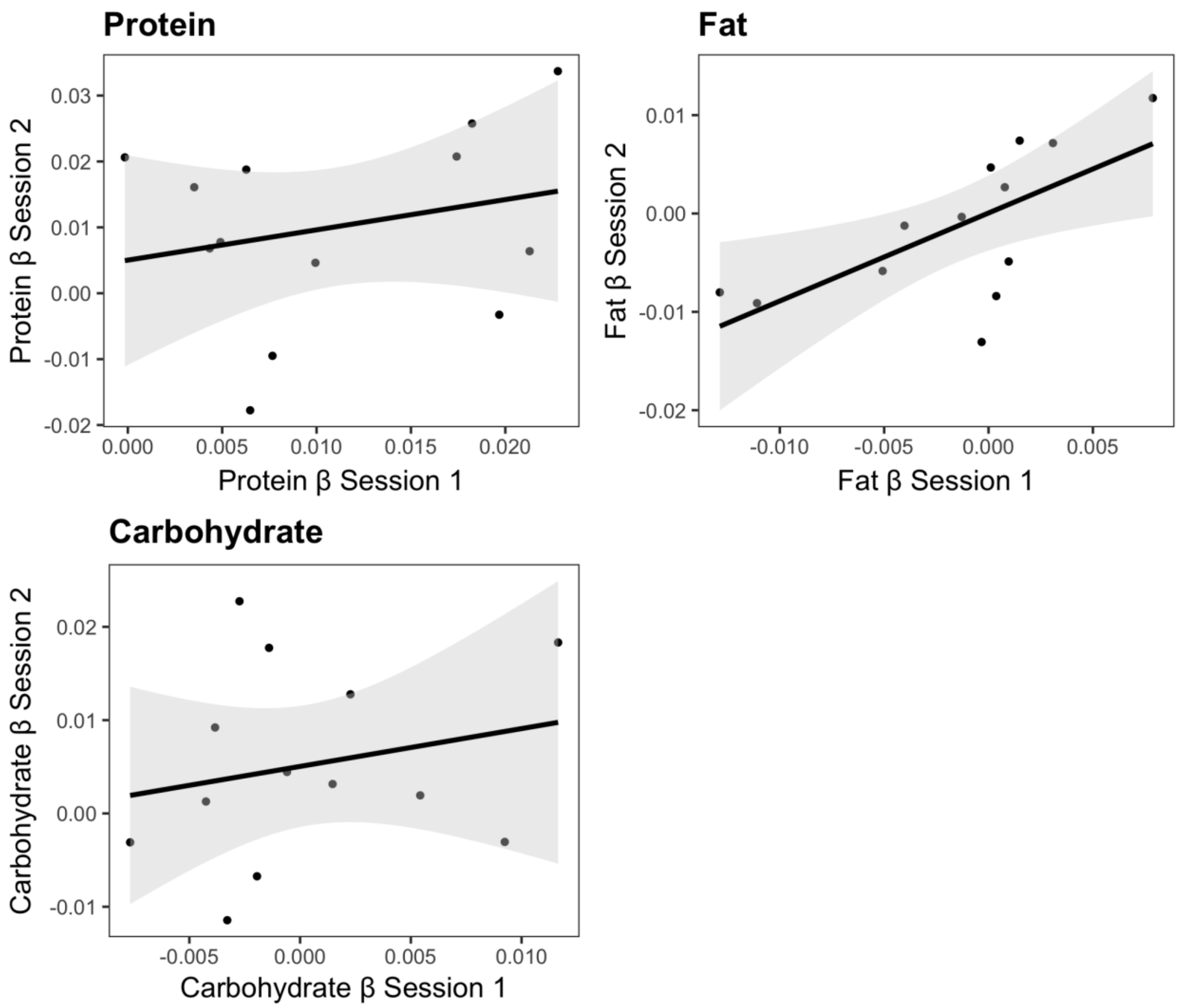

Figure 2-3 *Part 1: Relationship between participants' β coefficients for protein, carbohydrate and fat in session 1 and session 2 (shaded regions 95% CI)*

Pearson correlation indicates a significant relationship between participant's fat valuation in session 1 and in session 2 ($r = .658$, $p = .012$). This suggests that participant's fat valuations were consistent across both food lists. There was not a significant relationship between participant's β coefficients for protein in session 1 and session 2 (*r*= .247, *p*= .417) or between participant's β coefficients for carbohydrate in session 1 and session 2 (*r*=. 217, *p*= .476). This indicates that participant's protein and carbohydrate valuations were not the same across the different foods.

2.2.3 Discussion

The findings of the present study indicate that the novel, binary forced-choice task is an effective way of quantifying the relative value that people place on protein, carbohydrate and fat, calorie for calorie. There was a difference in the relative value that people placed on protein, carbohydrate and fat, suggesting that macronutrients are not valued equally. This supports the idea that humans have the ability to differentiate the macronutrient content of food. One possible explanation for the difference in value between protein and fat is the difference in the human body's ability to store macronutrients. The human body has limited storage capacity for protein whereas the body's fat reserves are virtually unlimited (Galgani & Ravussin, 2008). Therefore, the importance of maintaining protein intake is greater than the importance of maintaining fat intake and this translated in to individual's value of protein and fat.

The difference in value placed on carbohydrate and fat did not significantly differ in session 1 or session 2. This is unexpected due to the differences in energy reserves for fat and carbohydrate. As previously mentioned, fat reserves are almost unlimited, whereas the capacity to store carbohydrates as glycogen is limited (Acheson et al., 1988). Carbohydrates also provide essential energy for the central nervous system and as carbohydrate intake is tightly regulated, it was predicted that carbohydrates would be valued more than fat.

However, in the modern eating environment, both fat and carbohydrate have been negatively associated with health (Berthoud, Münzberg, Richards, & Morrison, 2012). Low-fat and low-carbohydrate diets have become popular as a dieting strategy for weight loss. In 2004, 26 million Americans were consuming a low- carbohydrate diet (Kemp, Burton, Creyer & Suter, 2007). Although, research has shown that low-nutrient products can have an undesirable effect and can increase intake and body weight (Wansink & Chandon, 2006). High-fat and high-carbohydrate food could be perceived as unhealthy and this may have influenced participant's food choices.

In general, previous findings have shown that energy dense, high-fat, high carbohydrate (sweet) foods are preferred, relative to high-protein, high-carbohydrate (starch) foods (Drewnowski, 1997). Whereas, the results from this study suggest that a calorie for calorie, fat was valued less than protein and carbohydrate. As the calories of fat increased in a food, people's relative value for fat decreased. Previous research has shown that high-calorie foods are preferred more than low-calorie foods. However, in the current experiment macronutrients are compared on a calorie for calorie basis, rather than comparing their absolute calorie content. In absolute measures, fat has 9 kcal per/g compared to protein and carbohydrate that have 4 kcal per/g. Analysing value on a calorie for calorie basis, means that each macronutrient is compared equally. It is also important to note that the binary forced-choice task is measuring macronutrient valuation rather than macronutrient preferences. The current research supports the idea that value and preference are different constructs.

Further research is required to investigate whether individual's macronutrient valuation remains consistent in different foods. Participant's protein and carbohydrate valuations were different for the food in session 1 and in session 2. There was not a relationship between participant's protein and carbohydrate valuations across session 1 and session 2. This could indicate that participant's macronutrient valuation is food specific; individuals might develop different valuations for different foods. The time of day could also influence macronutrient valuations and could explain why valuations for protein and carbohydrate were not correlated across the two test sessions. This idea is explored further in Chapter 3, where macronutrient valuations are compared at different meal times. Although, there are methodological concerns that could explain why participant's protein and carbohydrate valuations in session 1 did not correspond with the macronutrient valuation in session 2. The purpose of this experiment was to collect preliminary data to investigate the feasibility of a novel, binary-forced choice task to measure macronutrient valuation, and the sample size was relatively small (Button et al., 2013). Therefore, the results need to be replicated in a larger sample size and additional measures should be considered such as, healthiness and liking. Also, the large variability in macronutrient value between participants highlights the importance of considering individual differences in macronutrient valuation; this idea is explored in chapter 3 and 4.

To explore macronutrient valuation further, it is important to measure the consistency of participant's macronutrient valuations. Therefore, part 2 will investigate the test-retest reliability of the binary-forced choice task in measuring macronutrient valuation over two test sessions. If participants are consistent in the relative value that they place on protein, carbohydrate and fat then it would suggest that the binary forced-choice task is an efficient tool for measuring macronutrient value.

2.3 Part 2: Measuring the test-retest reliability of the binary forced-choice task

2.3.1 Methodology

The aim for this study was to determine whether participant's macronutrient valuations were consistent over a short period of time. Participants attended two, identical test sessions, which were one week apart. The participants completed a binary forced-choice task that determined whether the protein, carbohydrate or fat content of a food predicted food choice. Participants also completed measures of expected satiety, liking, perceived healthiness and familiarity.

The data collected for this experiment is also included in Chapter 4. The participants, measures and procedure are identical. The statistical analysis and the results are different in Chapter 2 and Chapter 4 and are relevant to the questions being investigated. The data was collected to investigate two separate research questions. The study protocol including hypotheses was preregistered with the Open Science Framework titled "Investigating individual's macronutrient valuation and whether this is influenced by perceived wellbeing." (https://osf.io/xhn9j/)

2.3.1.1 Design

The experiment was a repeated measures design. Participants completed the experiment twice, and test sessions were one week apart.

2.3.1.2 Participants

Based on data from the exploratory analysis in Chapter 3, a sample size was estimated. The exploratory analysis investigated the influence of perceived social status and macronutrient valuations. The effect size in this study was r= -0.4, considered to be medium according to Cohen's (1992) criteria. With an alpha = .05 and power = 80%, the projected sample size needed is approximately N = 85. A total of 92 participants were recruited**.** Of those 92, 8 participants were unable to attend their second test session and were removed from the data set. The remaining 84 participants (female= 57) had a mean age of 25.13 years (SD= 8.37, range= 19 - 71) and 73% of participants BMI scores were in the normal range (M= 23.03, SD= 4.21). Participants included staff and students from the University of Bristol (UK) and from the local Bristol area. All participants were reimbursed for their time with £15 for completing both test sessions and £10 for completing only the first test session.

Participants were excluded if they were vegan or vegetarian and if they had any food allergies or food intolerances.

2.3.1.3 Food stimuli

Images of 25 different foods were used in measures of food choice, expected satiety, perceived healthiness, liking and familiarity. To ensure that participants were familiar with the food, commonly consumed foods in the United Kingdom were selected (Henderson et al., 2002). The foods chosen had a range of macronutrient compositions and the relationship between macronutrients were not strong; protein and carbohydrate ($r = -0.363$, $p < .001$), fat and carbohydrate ($r = -0.324$, $p < .001$) and protein and fat ($r = 0.316$, $p < .001$). *Table 2-6* shows the macronutrient composition and the energy density of the 25 foods used in this experiment. Every food was photographed in 100-g portions on a white plate (255-mm diameter) using a high-resolution digital camera. The name of the food was presented in white font in the top-left-hand corner of every image. All food photographed was purchased from J. Sainsburys PLC and nutritional information was taken from the Sainsburys website and food packaging.

Table 2-6 *Macronutrient composition and energy density of the food stimuli*

	Nutrient (g) per 100g					*Energy (kcal) per 100g*			
Food	Fat	Carbohydrate	Protein	Sugar	Salt	Fat	Carbohydrate	Protein	Total
Avocado	19.5	1.9	1.9	0	0.5	175.5	7.6	7.6	190.7
Banana	0.5	23	1.2	20.9	0	4.5	92	4.8	101.3
Birds eye potato waffles	8.7	22	2.5	0.8	0.7	78.3	88	10	176.3
Blueberries	0.5	9.1	0.9	9.1	0	4.5	36.4	3.6	44.5
British ham slices	2.9	0.9	18.8	1.6	0.9	26.1	3.6	75.2	104.9
Broccoli	0.6	3.1	4.3	0	0	5.4	12.4	17.2	35
Chargrilled chicken	1.6	0.5	23.9	0.2	0	14.4	2	95.6	112
Chunky vegetables	1.7	5.9	1.1	5.2	0	15.3	23.6	4.4	43.3
Closed cup mushroom	0.5	0.5	1.8	0.5	0.5	4.5	2	7.2	13.7
Crumpets	1.1	42.6	6.6	3.9	1.12	9.9	170.4	26.4	206.7
Deli-style coleslaw	17	5.4	0.8	4.5	0.8	153	21.6	3.2	177.8
Heinz baked beans	0.2	12.9	4.7	5.0	0.6	1.8	51.6	18.8	72.2
King prawns	0.5	0.5	14.1	0.5	1.5	4.5	2	56.4	62.9

Large egg	9.6	0.5	14.1	0.4	0.5	86.4	2	56.4	144.8
Lincolnshire pork Sausages	17	8.4	14	0	1.3	153	33.6	56	242.6
Mild cheddar cheese	34.9	0.5	25.4	0	1.8	314.1	2	101.6	417.7
New York Bakery Co. plain Bagel	1.2	50.1	10.1	5.6	0.8	10.8	200.4	40.4	251.6
Penne pasta	0.7	32.5	5.1	0	0	6.3	130	20.4	156.7
Pink lady apples	0.5	11.8	0.5	11.8	0	4.5	47.2	2	53.7
Potato salad	13.1	10.4	1.8	0.7	0.7	117.9	41.6	7.2	166.7
Red seedless grapes	0.5	17	0.6	17.0	0	4.5	68	2.4	74.9
Smoked salmon	10.3	3.3	20.1	0	3.27	92.7	13.2	80.4	186.3
Sweet potato	0.5	18.9	1.1	8.0	0	4.5	75.6	4.4	84.5
Tuna (in spring water)	0.5	0.5	27	0.8	0.5	4.5	2	108	114.5
Unsmoked back bacon	13.8	1	25.8	0	3.8	124.2	4	103.2	231.4

Note: All food purchased was Sainsbury's own brand unless stated otherwise in the table

2.3.1.4 Measures of macronutrient value

Food choice:

Participants were presented with two food images, side by side on a computer monitor, in a binary forced-choice task. The instructions were "You will be shown two foods. Imagine this will be the only food you can eat between breakfast at 9am and dinner at 7pm, and you must only pick one of the two foods. You can take as little or as much as you want, and the amount is not limited to the portions shown." Participants used the left and right arrow keys to make their choice. All 25 food images were compared to each other in a random order, resulting in a total of 300 trials.

Expected Satiety:

The measure for expected satiety is identical to that in Part 1. All 25 images in this experiment were rated and were presented in a random order.

Perceived Healthiness:

Perceived healthiness was measured on a 100-mm VAS scale, anchored with 'Not at all healthy' and 'Extremely Healthy'. Participants were instructed to "Use the mouse to mark the line" in a place that represented their answer to the question "How healthy is this food?"

Familiarity:

Participants were presented with a food image and asked, "Have you eaten this food before?" Participants then had to click yes or no. This was repeated for all 25 test foods.

Liking:

Participants rated their liking for each food using a 100-mm VAS anchored with 'I hate it' and 'I love it'. The question "How much do you like the taste of this food?" was presented above the VAS and participants were instructed to 'Use the mouse to mark the line in an appropriate place'.

Hunger:

Participants were asked to use a 100-mm VAS to answer the question "How hungry are you right now?" anchored with 'Not at all hungry' and 'Very hungry'.

Questionnaires:

A demographic questionnaire was completed that recorded the participant's age, gender and experimental time slot preferences.

The Dutch Eating Behaviour Questionnaire (DEBQ) (Van Strien, Frijters, Bergers & Defares, 1986) is a measure to assess an individual's dietary behaviour. The questionnaire included 33 questions that are rated on a scale of 1- 5 (1- Never; 5- Very Often). Participants answered the questions in relation to whether each item is true to themselves. Each question is grouped in to one of three sub-scales; emotional eating, restraint and external eating. The scores for each subscale are then averaged to produce a mean score for each subscale. Higher scores indicate greater dietary restraint, emotional eating and external eating.

2.3.1.5 Procedure

Participants attended two test sessions, one-week apart at exactly the same time of day. A pre-session questionnaire was completed online before attending the test session to record participant demographics. On arrival at the laboratory, each participant read through an information sheet and signed a consent form before beginning. A consent form was completed in both test sessions. The binary forced-choice task was completed first, followed by measures of expected satiety, perceived healthiness, familiarity and liking. Height and weight were recorded at the end of the test session. Participants were given a debrief at the end of the second test session and reimbursed for their time. All measures were completed in the first and second test session. This experiment was approved by the Science Faculty Ethics Committee, University of Bristol (52163).

2.3.1.6 Statistical Analysis

In order to assess the test-retest reliability of the task, separate correlation coefficients (Pearson's) were calculated for each macronutrient β coefficient. A strong correlation coefficient would suggest that participant's values of protein, carbohydrate, and fat remained stable across the two test sessions. The statistical analyses for part 2 are identical to the statistical analyses conducted in part 1.

2.3.2 Results

2.3.2.1 Participant's demographics

Participant characteristics are summarised in Table 2-7, include age, hunger ratings, BMI and the three DEBQ subscales; emotional eating, restraint and external eating.

Table 2-7 *Participant's demographics*

	M	SD	Range
Age (years)	25.1	8.4	19 - 71
BMI	23.0	4.2	14.7 – 39.7
Hunger (mm)	55.6	1.8	0 – 100
DEBQ Emotional Eating	2.4	0.8	1.0 – 4.6
DEBQ Restraint	2.4	0.7	1.0 – 3.9
DEBQ External Eating	3.3	0.6	1.9 – 4.8

Table 2-8 displays the percentage of participants familiar with the test foods and the liking, expected satiety and perceived healthiness ratings, averaged across participants. The majority of participants were familiar with all of the test foods. The mean liking ratings of the foods ranged from 38.83 (coleslaw) to 92.67 (banana).

Table 2-8 *Mean (M), standard error (SD) and range of liking, expected satiety and perceived healthiness ratings and the percentage of familiarity of the test foods*

Food	Liking (mm)			Expected Satiety (kcal)			Perceived Healthiness (mm)			% of participants familiar with the test foods
	M	SD	Range	M	SD	Range	M	SD	Range	
Apple	74.5	18.8	24 - 100	146.8	109.8	20 - 660	87.6	10.5	60 - 100	100
Avocado	63.2	30.9	0 - 100	198.1	107.3	60 - 440	83.6	13.0	45 - 100	94
Bacon	71.5	25.9	0 - 100	282.4	128.4	20 - 660	16.3	17.4	0 - 92	98
Bagel	68.8	21.3	3 - 100	266.4	131.3	20 - 740	32.1	16.9	0 - 75	98
Baked beans	55.5	26.1	0 - 99	178.6	118.4	20 - 700	48.6	21.3	0 - 97	95
Banana	92.7	18.5	0 - 100	165.4	86.9	20 - 400	85.8	12.6	36 - 100	99
Blueberries	75.3	22.3	14 - 100	142.4	102.5	20 - 560	90.9	11.9	28 - 100	98
Broccoli	65.6	26.2	0 - 100	137.4	73.1	20 - 400	95.0	6.2	66 - 100	98
Cheese	73.1	23.9	0 - 99	227.1	113.7	40 - 680	30.9	19.2	0 - 92	100
Chicken	77.3	17.1	21 - 100	218.8	119.7	60 - 760	71.7	18.8	19 - 99	99
Coleslaw	38.8	29.7	0 - 95	148.7	113.4	20 - 720	30.9	19.2	0 - 89	82
Crumpets	66.1	22.2	4 - 100	239.5	98.6	20 - 540	30.5	18.1	0 - 92	92

Egg	66.5	26.9	2 - 99	183.5	90.8	20 - 460	76.4	14.6	31 - 99	99
Grapes	81.1	18.3	7 - 100	99.8	75.9	20 - 420	83.7	15.3	30 - 100	99
Ham	54.8	24.4	0 - 98	200.5	98.7	40 - 420	38.1	24.9	0 - 99	99
Mushroom	63.4	27.3	0 - 100	142.4	103.2	20 - 520	83.8	14.0	19 - 99	99
Pasta	70.1	21.9	12 - 99	231.3	105.1	20 - 660	44.7	17.9	7 - 98	100
Potato salad	52.9	24.9	0 - 99	169.2	91.2	20 - 580	32.7	17.8	2 - 82	95
Potato waffles	63.4	25.8	1 - 99	214.4	96.7	20 - 460	21.1	13.9	0 - 50	90
Prawns	67.4	26.7	0 - 99	193.2	79.7	60 - 420	70.1	17.3	20 - 99	96
Sausages	69.3	25.0	0 - 100	218.8	98.6	60 - 460	20.9	15.4	0 - 63	100
Smoked salmon	73.6	25.1	0 - 100	204.7	106.9	60 - 700	71.0	17.0	16 - 99	94
Sweet potato	77.9	19.5	8 - 100	232.0	95.3	20 - 500	74.0	18.0	13 - 99	96
Tuna	60.7	26.0	0 - 99	211.3	95.1	60 - 480	73.4	16.8	27 - 98	94
Vegetables	74.4	8.0	5 - 99	149.9	82.0	20 - 380	83.4	13.7	49 - 99	99

2.3.2.2 Is macronutrient valuation consistent across test sessions?

The mean β coefficients and standard error values for session 1 and session 2 are displayed in Table 2-9. As in part 1, the β coefficients are used to quantify the value that is placed on protein, carbohydrate and fat, calorie for calorie. The β coefficient refers to the odds of choosing the right-hand food when the right –hand food contains 1kcal/ 100g more protein than the left-hand food. Across participants, in both session 1 and session 2, protein had the largest β coefficient followed by carbohydrate, then fat; proposing a macronutrient valuation hierarchy of protein > carbohydrate > fat.

Table 2-9 *Summary of the binary logistic regression analysis for variables predicting food choice in session 1 and session 2*

	Session 1		Session 2	
	β	SE	β	SE
Protein	0.0068	0.0012	0.0061	0.0011
Carbohydrate	0.0054	0.0011	0.0047	0.0012
Fat	-0.0015	0.0005	-0.0016	0.0005

Note: β = unstandardized regression weight, SE = standard error of β

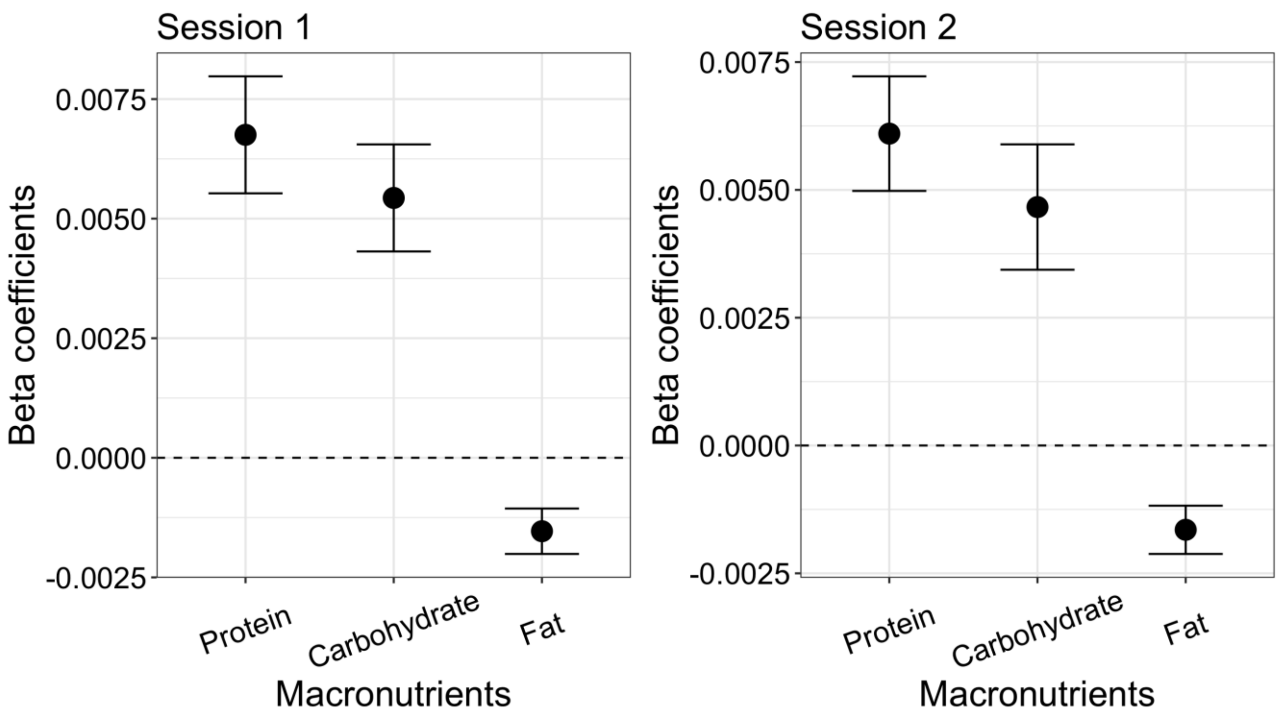

Figure 2-4 *Averaged multilevel binary logistic regression β coefficients for protein, carbohydrate and fat in session 1 and session 2.*

For session 1, a one-way ANOVA was conducted to compare the differences in the amount of value placed on protein, carbohydrate and fat. The results indicate that there was a significant difference in the β coefficients for protein, carbohydrate and fat, $F(2,249) = 20.00$, $p < .001$. A Tukey test specified that protein was valued significantly more than fat ($p < .001$), and that carbohydrate was valued significantly more than fat ($p < .001$). There was no significant difference in the valuation of protein compared to carbohydrate ($p = .617$).

For session 2, there was a significant difference in valuation between macronutrients determined by a one-way ANOVA, $F(2,249) = 17.09$, $p < .001$. A Tukey test indicated a significant difference in the valuation of protein compared to fat ($p < .001$) and in the valuation of carbohydrate compared to fat ($p < .001$). There was no significant difference in the amount of value placed on protein compared to carbohydrate ($p = .566$). In other words, in session 1 and 2, protein and carbohydrate are valued significantly more than fat, but protein and carbohydrate are valued to a similar extent.

Figure 2-5 shows the relationship between each participant's β coefficient for protein, carbohydrate and fat in session 1 and session 2. Pearson's correlations indicate that session 1 and session 2 β coefficients are strongly correlated for protein (r= 0.84, p< .001), carbohydrate (r= 0.89, p< .001) and fat (r= 0.90, p< .001). Participant's macronutrient valuation remained constant over the two test sessions suggesting, that there is good test-retest reliability for the binary forced-choice measure of macronutrient valuation.

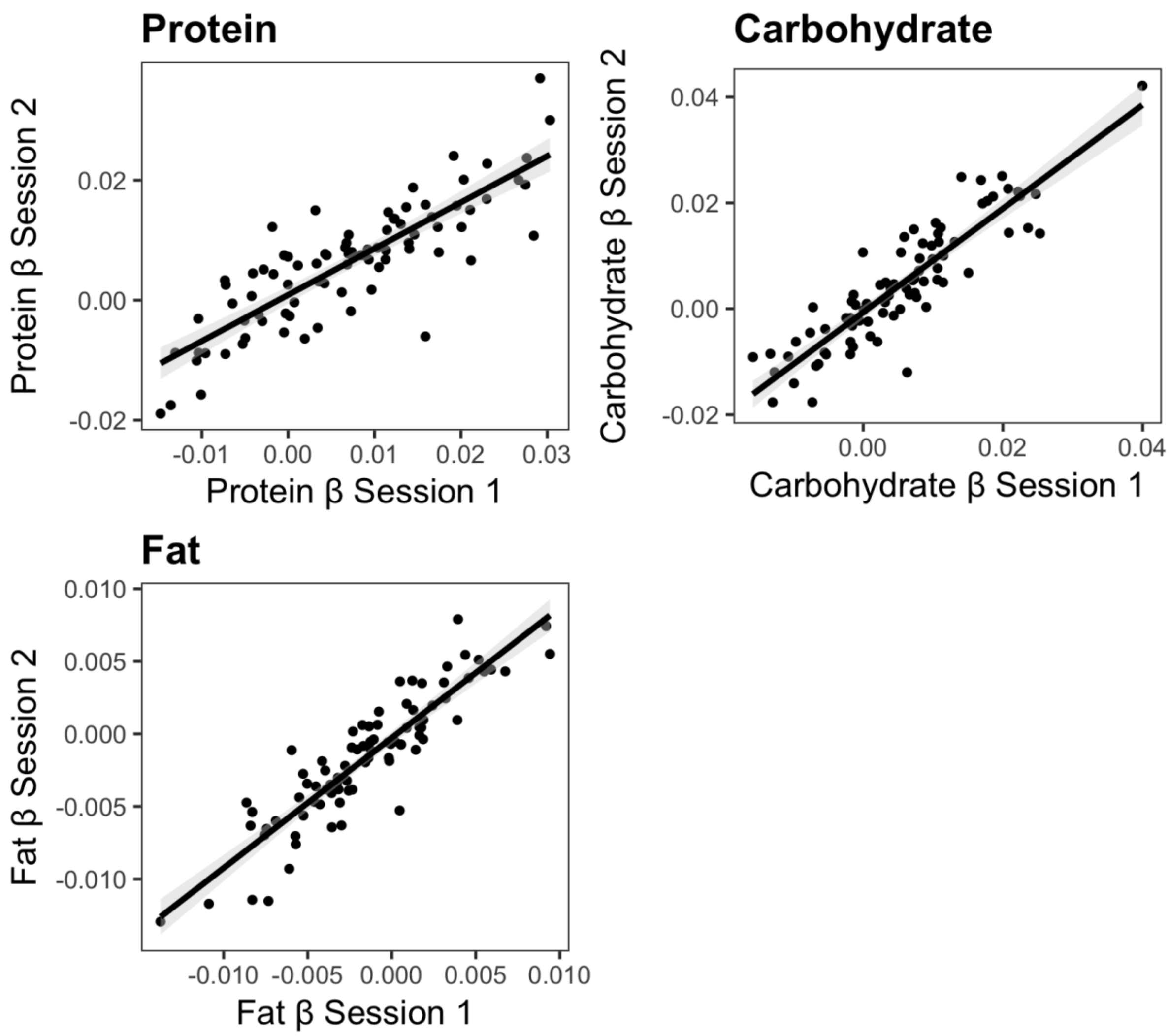

Figure 2-5 *Part 2: Relationship between participant's multilevel binary logistic regression β coefficients for protein, carbohydrate and fat in session 1 and session 2*

2.3.3 Discussion

The results from this study indicate that the binary forced-choice task used to measure macronutrient valuation has good test-retest reliability. There was a strong relationship between participant's relative value for protein, carbohydrate and fat in session 1 and in session 2. Therefore, indicating that participant's macronutrient valuations remained constant over a one- week period. This suggests that in the short- term, participant's relative value for protein, carbohydrate and fat do not change. Further research is needed to explore individual's macronutrient valuation over a long-term period. Based on Hechter's (1993) argument that values are stable, it is assumed that, among adults, an individual's relative value for protein, carbohydrate and fat, in this specific food list, would remain stable over a long-term basis.

Previous research investigating macronutrient preferences (de Bruijn et al., 2017) has grouped foods into macronutrient categories, and this means that foods in the same group are not compared. In order to calculate a relative value for protein, carbohydrate and fat it was important for all foods to be compared to one another. All foods are made up of a percentage of protein, carbohydrate and fat and are very rarely isolated to one nutrient. Therefore, the differences between the macronutrient content of foods are an important determinant of food choice. For example, prawns and eggs both have a high protein content (14.1g of protein per/100g), but they have very different fat contents. Prawns have 0.5g of fat per/100g whereas eggs have 9.6g per/100g. The choice between prawns and eggs provides information about whether fat is valued more (eggs) or less (prawns), when protein and carbohydrate are held constant. If eggs are selected over prawns, then the food with less fat is rejected and this is evidence for fat valuation. Therefore, a strength of the binary forced-choice task is that subtle shifts in macronutrient value are detected because participants could not skip a trial, and this ensured that each food was compared to every other food.

Another strength of the binary forced-choice task design is that it is easy to complete. The instructions are straight-forward, and the computer-based task can be easily transported. The test foods can also be changed to match commonly consumed foods in the country of completion, therefore making it a universally adaptable tool. This is useful for future research that can investigate macronutrient valuation in different ages and in people with different medical needs. For example, elderly people are at a greater risk of sarcopenia (Doherty, 2003) and therefore exploring macronutrient valuation in an elderly population might help guide dietary advice.

There were 25 food images included in this study compared to the 21 and 20 food images used in part 1, session 1 and part 1, session 2, respectively. The number of food images included in the current experiment was increased because it could be argued that the more foods included in the forced-choice task then the better representation of macronutrient value. However, it is important to note that as the number of foods increases so does the number of comparative trials. Part 2 included 300 trials compared to 210 in part 1, session 1 and 190 trials in part 1, session 2. The more trials there are, the higher the risk of participant's becoming tired or bored during the task. This can lead to mental fatigue effects and could result in increased variance in results that do not represent the intended measure (Gonzalez, Best, Healy, Kole, & Bourne, 2011). Therefore, in forced-choice tasks it is important to consider the trade-off between increasing the amount of food images and the tediousness of the task.

In conclusion, both part 1 and part 2 suggest that macronutrients are not valued equally, and therefore this supports the idea that humans have the ability to differentiate the macronutrient content of food. The binary forced-choice task is a feasible and effective tool for assessing macronutrient valuation. The test-retest reliability was strong and suggests that individual's macronutrient valuation does not change over a short-period of time. Further research is needed to explore whether the relative value that people place on macronutrients is food specific or consistent across food groups. In Chapter 3, macronutrient valuation is investigated across different meal times.

Chapter 3 Investigating macronutrient valuations in lunchtime foods and breakfast cereals

3.1 Introduction

In Chapter 2, averaged across all participants, protein was valued significantly more than carbohydrate and fat, on a calorie for calorie basis. Participants varied considerably in the relative value that was placed on protein, carbohydrate and fat. Within participants, across two-test sessions, macronutrient value remained stable, indicating a strong test retest-reliability. To further investigate macronutrient valuation, in the current chapter, an additional food list was added that included breakfast cereals. The aim was to determine whether individual's macronutrient valuations remained similar across two different meal times; lunchtime and breakfast.

People regularly refer to breakfast as the most important meal of the day, however, in the fast-paced, modern environment; people often skip breakfast (Siega-Riz, Popkin, & Carson, 1998). Research suggests that the dietary habit of skipping breakfast is associated with increased BMI and obesity (Deshmukh-Taskar et al., 2010). It is important to eat breakfast because eating a large percentage of our daily energy intake in the morning is associated with lower energy intake over the entire day (de Castro, 2004). People who eat breakfast are more likely to make healthy food choices and have a good quality diet (Reeves, Halsey, McMeel, & Huber, 2013). The opposite association is seen when the majority of our energy intake is consumed in the evening. If energy intake is relatively high in the evening, then daily energy intake overall tends to be higher (Wang et al., 2014). This effect could be because the satiating effect of food decreases throughout the day therefore, portion sizes tend to increase with each meal and subsequently energy intake increases (de Castro, 2004).

In Western countries, carbohydrate intake is usually greatest at breakfast and intake is lower during lunchtime and dinner. Whereas, protein and fat intake are usually lower at breakfast and intake increases during lunchtime and dinner (Dwyer et al., 2001). Macronutrient intake differs between each meal and de Castro (2007) found the macronutrient intake in the morning could influence macronutrient intake in the evening. After consuming a large proportion of daily energy as carbohydrate, protein or fat in the morning, over the rest of the day the amount of energy as carbohydrate, protein or fat, respectively, is reduced. For example, carbohydrate intake in the morning reduces carbohydrate intake throughout the rest of the day. This pattern of nutrient intake is the same for protein and fat.

In a study by Teff, Young and Blundell (1989), participants consumed either a high-protein or a high-carbohydrate breakfast, with similar sensory properties, followed by an ad-libitum lunch. The macronutrient composition of the participant's breakfast did not influence their selection of macronutrients at lunch. Specifically, participants did not reduce their protein or carbohydrate intake at lunchtime to balance out their macronutrient intake at breakfast. There was one exception, participants who had eaten the high- carbohydrate breakfast selected less apple. The apple was the only food available at lunchtime with a 100% carbohydrate content (protein 0 g/g food, carbohydrate 0.14 g/g food). These results question the findings of de Castro (2007) and suggest that the underlying mechanism used to regulate the association between macronutrient intake in the morning and macronutrient intake throughout the day could be very specific. Carbohydrate intake in the morning might only affect carbohydrate intake later in the day when the food item consumed is solely energy from carbohydrate.

As previously mentioned in Chapter 1, in developed countries, people with lower socioeconomic status tend to have a higher BMI than people with higher socioeconomic status (Ball & Crawford, 2005). Recently, the relationship between perceived social status and food choice has been explored (Goodman et al., 2003).Perceived social status is defined as an individual's perception of one's social standing in society, in relation to others (Adler et al., 2000). Research suggests that perceiving oneself to have low social standing in one's community is associated with an increased risk of obesity (Tang, Rashid, Godley, & Ghali, 2016). Cheon and Hong (2017) experimentally provoked feelings of high or low perceived social status. Their results demonstrated that people who experienced low perceived social status increased their preference of energy dense foods in a laboratory setting. This indicates that low perceived social status might promote the preferential selection of foods with a particular dietary composition and this might indicate a specific tendency to show a different pattern of macronutrient valuation.

The aim of the current study was to investigate whether individual's macronutrient valuations remains similar in lunchtime foods and breakfast cereals. It was predicted that individual's value for protein, carbohydrate and fat in the lunchtime foods would correspond with their value in the breakfast cereals. If their macronutrient valuations were similar in the lunchtime foods and breakfast cereals, then this would suggest that across two different meal times macronutrient valuations are consistent.

Additionally, fixed and variable portion size conditions were included to identify whether participants were aware of the portion size on offer and to determine whether the macronutrient valuation hierarchy, seen in the previous chapter, would be preserved across the two different portion size conditions. It was predicted that the order of value, such that protein is valued more than carbohydrate and fat on a calorie for calorie basis, is consistent across the fixed and variable portion size conditions.

Measures of socioeconomic status and perceived social status were included to explore the possibility that perceived social status influences macronutrient valuation, independent of socioeconomic status. People with lower perceived social status might show specific patterns of valuation for protein, carbohydrate and fat. There are no previous studies that have explored the relationship between perceived social status and individual's macronutrient valuations.

3.2 Methodology

3.2.1 Design

The experiment included two food lists, the first was lunchtime foods and the second was commonly consumed UK breakfast cereals. There were two portion size conditions; fixed portion size and variable portion size. Participants were randomly allocated to the fixed or variable portion size condition. Participants were assigned to a condition based on the test-session they attended. Both portion size conditions included identical lunchtime and breakfast food lists.

3.2.2 Participants

A sample size estimation was performed, based on Cohen's (1992) criteria for an ANOVA analysis comparing 3 groups (protein, carbohydrate and fat). With an alpha of .05 and power achieved 80%, the sample size needed for a medium effect size was approximately N= 52, in each mealtime condition. 116 participants were recruited (97 females) and one participant was removed from the analysis because they informed the experimenter that they did not eat animal-based products. The remaining 115 participants had a mean age of 19.77 years (*SD*= 2.10, range= 18.00 – 33.00) and a mean BMI of 21.95 (*SD*= 3.25, range= 16.07 – 35.60). There were 57 participants in the fixed portion size condition and 58 in the variable portion size condition. All participants were recruited using the University of

Bristol, Experimental Psychology, experimental hours scheme. All participants were University of Bristol, Experimental Psychology students and received one experimental hour's credit for their participation. Due to the inclusion of animal-based foods, vegetarians, vegans and people

with a food intolerance or a food allergy were excluded from the recruitment process. This study was conducted according to the University of Bristol ethical guidelines and was given approval from the Science Faculty Ethics Committee, University of Bristol (45742).

3.2.3 Food stimuli

All food was purchased from J. Sainsburys PLC and nutritional information was obtained from food packaging and the Sainsbury's website.

<u>Lunchtime food:</u> The foods selected for the lunchtime food list were based on the two food lists used in Chapter 2, Part 1. The 17 lunchtime foods were chosen to have a range of macronutrient compositions and energy densities (see Table 3-1 for a full list of the nutritional information). The food was photographed in 100-g portions on a white plate (225-m diameter) using a high-resolution camera that was positioned at a 45-degree angle. The name of the food was presented in the top-left-hand corner of the image in white writing.

<u>Breakfast cereals:</u> All 16 breakfast cereals selected were UK brands (see *Table 3-2* for full list of nutritional information). The cereal was photographed in 50-g portions in a white bowl placed on top of a white plate. A glass jug filled with 100-ml of milk was presented in the background to recreate a realistic breakfast situation, as breakfast cereal in the UK, is commonly consumed with milk. The name and brand image for each breakfast cereal was presented in the top-left-hand corner. The brand image was included to make it clearer for participants to identify the cereal.

Table 3-1 *Macronutrient composition and energy density of the lunchtime food in g/100g and kcal/100g*

	Nutrient (g) per 100g					Energy (kcal) per 100g			
Food	Fat	Carbohydrate	Protein	Sugar	Salt	Fat	Carbohydrate	Protein	Total
Apple	0.5	12	0.5	0.5	11.8	4.5	48	2	54.5
Avocado	19.5	1.9	1.9	0	0.5	175.5	7.6	7.6	190.7
Bagel	1.3	48.9	10.3	0.8	5.6	11.7	195.6	41.2	248.5
Banana	0.5	23	1.2	0	20.9	4.5	92	4.8	101.3
Chicken	1.6	0.5	23.9	0.2	0	14.4	2	95.6	112
Coleslaw	17	5.4	0.8	0.8	4.5	153	21.6	3.2	177.8
Egg	9.6	0.5	14.1	0.4	0.5	86.4	2	56.4	144.8
Mackerel	22.2	0.5	20.8	1.5	0.5	199.8	2	83.2	285
Grape	0.5	15.4	0.5	0	17	4.5	61.6	2	68.1
Ham	2.3	1.7	19	1.6	0.9	20.7	6.8	76	103.5
Pasta	0.7	32.5	5.1	0	1.5	6.3	130	20.4	156.7
Potato salad	10.2	10.6	1	0.7	2.8	91.8	42.4	4	138.2
Prawn	0.5	0.5	14.1	1.5	0.5	4.5	2	56.4	62.9

Sausage	18.7	6.7	13	1.5	1.3	168.3	26.8	52	247.1
Tuna	0.5	0.5	27	0.8	0.5	4.5	2	108	114.5
Turkey	0.8	2.6	22.3	0.9	1.2	7.2	10.4	89.2	106.8

Note: All food stimuli were Sainsbury's own brand

Table 3-2 *Macronutrient composition and energy density of the breakfast food in g/100g and kcal/100g*

	Nutrient (g) per 100g					Energy (kcal) per 100g			
Food	Fat	Carbohydrate	Protein	Sugar	Salt	Fat	Carbohydrate	Protein	Total
Alpen	5.8	67	11	0.3	21	52.2	268	44	364.2
Branflakes	3.2	63	12	0.7	14	28.8	252	48	328.8
Cheerios	3.7	74	8.8	0.9	21	33.3	296	35.2	364.5
Coco Pops	2.5	85	5.5	0.8	30	22.5	340	22	384.5
Cornflakes	0.9	84	7	1.1	8	8.1	336	28	372.1
Crunchy Nut	5	82	6	0.8	35	45	328	24	397
Crunchy Nut Chocolate	17	67	8	0.8	31	153	268	32	453
Frosties	0.6	87	4.5	0.8	37	5.4	348	18	371.4
Fruit ‘N’ Fibre	5.6	67.5	9.3	1.0	24	50.4	270	37.2	357.6
Golden Nuggets	1.5	81.5	7.6	0.7	25	13.5	326	30.4	369.9
Jordan’s	14.9	66.5	8.1	0.3	20.8	134.1	266	32.4	432.5
Krave	15	69	7.1	1.0	28	135	276	28.4	439.4
Rice Krispies	1	87	6	1.1	10	9	348	24	381

Shreddies	1.7	71	11	0.1	0.7	15.3	284	44	343.3
Shredded Wheat	2.2	68	12	0.7	15	19.8	272	48	339.8
Weetabix	2	69	12	0.3	4.4	18	276	48	342

3.2.4 Measures

Food choice:

Participants completed a similar computer-based, binary forced-choice task as previously described in Chapter 2. The 17 lunchtime foods (136 trials) were presented with the instructions 'Imagine this will be the only food you can eat between breakfast at 9am and dinner at 7pm, and you must only pick one of the two foods.' The 16 breakfast cereals (120 trials) were presented with the instructions "You must pick one of these cereals to eat for breakfast and you can only pick one."

The portion size instructions presented with the lunchtime and breakfast food was specific to the fixed and variable portion size condition. Participant in the fixed portion size condition were informed that 'Only these portions are available.' Participant in the variable portion size condition were presented with the instructions 'You can take as little or as much as you want, and the amount is not limited to the portions shown.'

Expected satiety:

The measure for expected satiety is the same as in Chapter 2. However, the comparison food for the breakfast cereals was changed from 'Uncle Ben's classic pilau rice' to bananas as they are more comparable to breakfast cereals than rice. The plate of bananas could be increased or decreased in 20 kcal portions and ranged from 20 kcal to 800 kcal.

Hunger:

Hunger was measured on a 100-mm VAS anchored with "Not at all hungry" and "Extremely hungry". Participants were asked "How hungry are you now?" and use the mouse to click on the 100-mm line to answer the question.

Familiarity:

Participants were shown a food image and asked the question 'Have you eaten this food before?' Participants then clicked on the 'Yes' or 'No' button on screen before moving on to the next food. All lunchtime foods and breakfast cereals were presented.

Perceived Healthiness:

Participants rated their perceived healthiness for all test foods using a 100-mm VAS. The VAS was anchored 'Not at all healthy' and 'Extremely Healthy'. The question 'How healthy is this food?' was presented above a food image. Participants then used the mouse to put a mark on the line that indicated how healthy they perceived each food to be.

Questionnaires:

Participants completed a demographics questionnaire that included measures of age, gender and employment. Participants also completed the Dutch Eating Behaviour Questionnaire (van Strien, Frijters, Bergers, & Defares, 1986) previously described in Chapter 2, part 2.

As an exploratory measure of participant's perceived social status, the MacArthur Scale of Subjective Social Status (Adler et al., 2000) was included. Participants in the variable portion size condition completed the MacArthur Scale of Subjective Social Status. Participants were presented with an image of a ladder and are asked to "Think of this ladder as representing where people stand in their communities. People define community in different ways; please define it in whatever way is most meaningful to you. At the top of the ladder (10) are people who have the highest standing in their community and at the bottom (1) are people who have the lowest standing in their community. Where would you place yourself on this ladder?" Participants are then asked to mark an "X" on the rung that represents where they think they stand in the community, relative to other people. There are 10 rungs on the ladder; 10 represents a high perceived social status and 1 represents a low perceived social status.

Participant's postcodes were also recorded as a control measure of socioeconomic status. Postcodes were used to measure participants Index of Multiple Deprivation (IMD) (Office of National Statistic Indices of Multiple Deprivation, 2010). The IMD is a UK government study of deprived areas in England and each postcode is given an IMD score and grouped in to IMD quintiles. The scores range from less than or equal to 8.49 representing the least deprived areas (quintile group 1) and more than or equal to 34.18 representing the most deprived areas (quintile group 5).

3.2.5 Procedure

Participants attended the laboratory for a one-hour test session. All participants were given an information sheet and signed a consent form before beginning the experiment. Participants were randomly allocated to a portion size condition before they arrived at the laboratory so that the related tasks could be loaded on the computer. Participant's age, height, weight and gender were recorded at the start of the test session. Participants completed the following measures on the computer; food choice, hunger, familiarity, expected satiety and perceived healthiness, for both the lunchtime and breakfast foods. The order of lunchtime and breakfast foods was counterbalanced. The DEBQ and MacArthur Scale of Subjective Social Status were completed in paper form and postcodes were recorded after the computer-based tasks. Before leaving, all participants were debriefed and given the experimenters contact details for any further questions.

3.2.6 Statistical Analysis

All data analyses were performed using R software (R Development Core Team, 2010) and the significance value was set at $p < .05$. The protein, carbohydrate and fat content kcal /100g was calculated for the lunchtime and breakfast foods. For each binary forced-choice trial, a separate ‘difference score’ was calculated (right-hand food – left-hand food) for protein, carbohydrate and fat.

Individual’s macronutrient valuations

For each participant, in both the fixed and variable portion size condition, a separate binary logistic regression analysis was run to investigate the relative value placed on protein, carbohydrate and fat, calorie for calorie, in the lunchtime and breakfast foods. Food choice was entered in to the model as the outcome variable and the protein, carbohydrate and fat difference scores were entered as predictors. The β coefficients were used to quantify the relative value placed on protein, carbohydrate and fat, calorie for calorie, in lunchtime and breakfast foods, separately.

One-way analysis of variance (ANOVA) tests were conducted to determine whether there was a difference between the value placed on protein, carbohydrate and fat in the breakfast food and in the lunchtime foods, separately. This was repeated in the fixed and variable portion size conditions. Individual’s protein, carbohydrate and fat β coefficients were used as the outcome variables and the macronutrient difference scores were used as the predictors. A Tukey test was used to investigate where the difference in value occurred between protein, carbohydrate and fat.

Pearson’s correlations were conducted to investigate whether there was a relationship between the relative value placed on protein, carbohydrate and fat in breakfast and lunchtime foods. The average β coefficient for protein in breakfast foods was correlated with the average β coefficient for protein in the lunchtime foods. This was repeated for carbohydrate and fat. The correlation coefficients indicate whether participant's macronutrient value is similar across two different meal times.

Differences in value in the fixed and variable portion size condition

Independent sample t-tests were used to investigate whether macronutrient valuation differed between the fixed and variable portion size conditions. The protein, carbohydrate and fat β coefficients in the fixed portion size condition were compared to the protein, carbohydrate and fat β coefficients in the variable portion size condition. Only the macronutrient β coefficients for the lunchtime foods were included in this analysis.

Perceived social status and macronutrient valuation

An exploratory analysis using a multilevel binary logistic regression was conducted to investigate whether individuals with lower perceived social status indicated a specific tendency to value protein, carbohydrate and fat differently to those with a higher perceived social status. In the task, each food was compared to all other foods and therefore, the assumption of independence of ratings is violated. To account for participant variability, trials were nested within participants in a multilevel logistic regression model. Food choice was used as the outcome variable in a multilevel binary logistic regression and interaction terms were entered between each macronutrient difference score and perceived social status and IMD quintile score. Participant's index of multiple deprivation quintile score was reversed so that a low quintile score represented a low socioeconomic status score and these scores were added in to the model as a control variable. The interaction terms showed whether an increase in protein difference, carbohydrate difference and fat difference was a stronger predictor of food choice and therefore higher valuation, in people with lower or higher perceived social status.

3.3 Results

3.3.1 Participant's demographics

Table 3-3 summarised participant characteristics in the fixed and variable portion size conditions. There is a slightly higher percentage of females in the fixed portion size condition, but the other participant characteristics are well-matched.

Table 3-3 *Participant's demographics*

	Fixed portion size n= 57			Variable portion size n= 58		
	M	SD	Range	M	SD	Range
Gender (%Female)	93			74		
Age (years)	19.9	2.2	18– 29	19.7	2.0	18 – 33
BMI	22.3	3.8	17.7 – 35.6	21.6	2.6	16.1 – 31.1
Hunger (mm)	45.6	2.4	0 – 90	44.6	2.3	0 – 90
DEBQ restraint	2.6	0.9	1.0 – 4.9	2.5	0.9	1.0 – 4.5
DEBQ emotional eating	2.9	0.8	1.3 – 4.9	2.7	0.6	1.3 – 3.8
DEBQ external eating	3.5	0.6	1.8 – 5.0	3.5	0.6	2.5 – 5.0

3.3.2 Familiarity

Table 3-4 *Percentage (%) of participants familiar with each of the lunchtime and breakfast foods in the fixed and variable portion size condition*

	Fixed	Variable		Fixed	Variable
Breakfast cereals	% Familiar	% Familiar	Lunchtime Foods	% Familiar	% Familiar
Alpen	64.9	69.0	Apple	100.0	100.0
Branflakes	68.4	70.7	Avocado	88.5	91.4
Cheerios	86.0	93.1	Bagel	100.0	100.0
Coco Pops	96.5	98.3	Banana	98.1	100.0
Cornflakes	93.0	98.3	Chicken	100.0	100.0
Crunchy Nut	61.4	63.8	Coleslaw	88.5	87.9
Crunchy Nut Choc	84.2	82.8	Egg	98.1	96.6
Frosties	84.2	94.8	Mackerel	59.6	81.0
Fruit N Fibre	52.6	63.8	Grape	100.0	100.0
Golden Nuggets	54.4	60.3	Ham	96.2	100.0
Jordan's	57.9	65.5	Pasta	100.0	100.0
Krave	57.9	63.8	Potato salad	92.3	86.2
Rice Krispies	89.5	93.1	Prawn	90.4	91.4
Shreddies	68.4	72.4	Sausage	100.0	98.3
Shredded Wheat	73.7	82.8	Tuna	90.4	96.6
Weetabix	91.2	94.8	Turkey	100.0	100.0
			Vegetables	96.2	96.6

The majority of participants were familiar with all of the lunchtime foods. The least familiar food was mackerel; 81% of participants in the variable condition and only 59.6% of participants in the fixed condition were familiar with mackerel. 100% of participants, in both conditions were familiar with apple, chicken, bagel, turkey, pasta and grapes. The breakfast cereals were less familiar than the lunchtime foods.

3.3.3 Socioeconomic measures

Participants in the variable portion size condition completed the McArthur Scale of Subjective Social Status to investigate the extent to which macronutrient valuations differ for individuals with higher and lower perceived social status. Table 3-5 displays the mean, standard deviation and range of IMD scores and IMD quintiles and the MacArthur Scale perceived social status scores.

Table 3-5 *Mean (M), standard deviation (SD) and range for participants Index of Multiple Deprivation (IMD) scores, quintile group and MacArthur Scale of perceived social status scores*

Measure	Mean	SD	Range
Age (years)	19.3	0.8	18 – 21
Gender (% Female)	59		
IMD score (1 – 42)	15.2	1.6	1.6 – 39.1
IMD quintile (1 – 5)	4.2	0.7	1 – 5
Perceived social status (10 ladder rungs)	5.0	1.3	2 – 7

The IMD tool used to convert postcodes to IMD scores only accepted English postcodes and therefore 20 participants who had postcodes from outside England did not have IMD scores. Also 16 participants either did not know their postcode or recorded it incorrectly. Therefore, a total of 22/58 participants were included in the analysis. All of the 22 participants were students, 16 were unemployed, and 6 had part-time jobs.

3.3.4 Macronutrient valuation in breakfast and lunchtime foods

The averaged β coefficients for protein, carbohydrate and fat were used to quantify the relative value that participants placed on each macronutrient, on a calorie for calorie basis. In each model, every β coefficient refers to the odds of choosing the right-hand food when the right –hand food contains 1kcal/ 100g more protein than the left-hand food. These β coefficients quantify macronutrient valuation. The β coefficients for carbohydrate and fat can be interpreted in the same way.

Figure 3-1 shows the mean β coefficients and standard error bars for protein, carbohydrate and fat, in the *fixed* lunchtime foods and breakfast foods. The β coefficients can be ranked in a macronutrient valuation hierarchy. In both the fixed lunchtime and fixed breakfast session, the order of value is represented as protein > carbohydrate > fat. In other words, across participants, calorie for calorie protein is valued to a greater extent than carbohydrate and fat and carbohydrate is valued to a greater extent than fat.

Figure 3-1 displays the mean β coefficients and standard error bars for protein, carbohydrate and fat in the *variable* lunchtime foods and breakfast foods. The results indicated a macronutrient valuation hierarchy as protein > carbohydrate > fat in the lunchtime, variable portion size condition. Calorie for calorie, protein is valued the most, followed by carbohydrate and the least valued macronutrient is fat. Regarding the variable, breakfast cereals the order of macronutrient valuation is fat > carbohydrate > protein. This is the opposite order of value compared to the lunchtime foods.

Table 3-6 *Summary of multilevel binary logistic regression for variables predicting food choice – lunchtime foods and breakfast cereals*

	Fixed portion size				Variable portion size			
	Lunchtime		Breakfast		Lunchtime		Breakfast	
	β	SE	β	SE	β	SE	β	SE
Protein	0.0172	0.0028	0.0147	0.0171	0.0110	0.0019	-0.0081	0.0215
Carbohydrate	0.0130	0.0016	0.0088	0.0063	0.0085	0.0015	0.0014	0.0074
Fat	0.0003	0.0008	0.0078	0.0028	-0.0018	0.0005	0.0056	0.0030

Note – β = unstandardized regression weight, SE = standard error of β

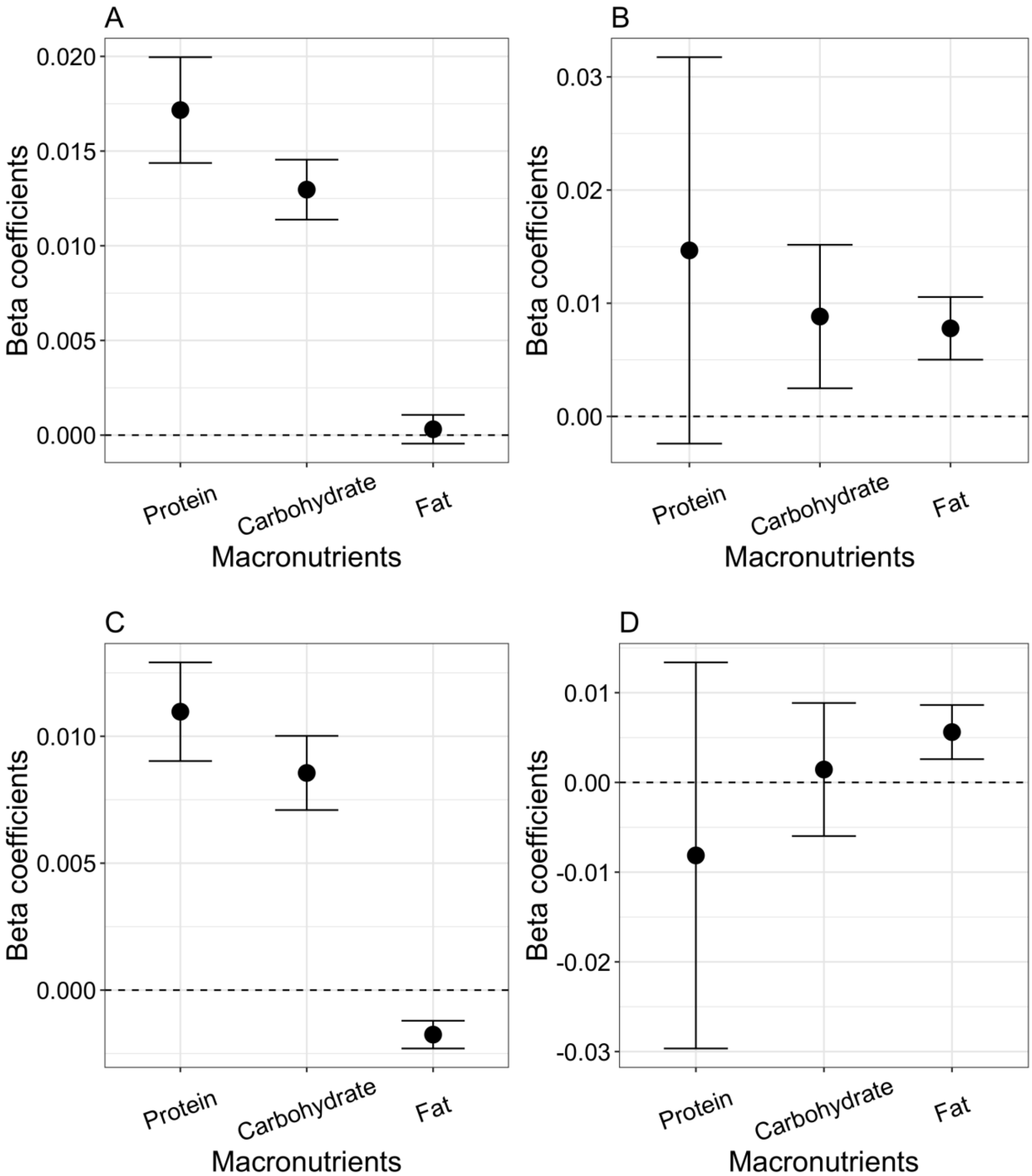

Figure 3-1 *Mean β coefficients (+/- 95% confidence interval) for predictors of choice in the fixed and variable portion size conditions. For the fixed portion size condition, the β coefficients for macronutrient value in the lunchtime foods are shown in panel A and breakfast foods in panel B. For the variable portion size condition, the β coefficients for macronutrient value in the lunchtime foods are shown in panel C and breakfast foods in panel D.*

Is the same amount of value placed on each macronutrient?

One-way ANOVA tests confirmed that β coefficients differed across macronutrients in the fixed lunchtime foods, $F(2, 168)=21.21$, $p< .001$ and variable lunchtime foods, $F(2, 171)=22.06$, $p< .001$. In other words, participants placed a different amount of value on each macronutrient. A Tukey test showed that in the fixed, lunchtime foods, protein was valued significantly more than fat ($p< .001$) and that carbohydrate was valued significantly more than fat ($p< .001$). There was not a significant difference in the value placed on carbohydrate and protein ($p= .266$). A second Tukey test showed the same pattern of value for the variable portion size condition, lunchtime foods. Protein was valued significantly more than fat ($p< .001$) and carbohydrate was valued significantly more than fat ($p< .001$) but there was not significant difference in the value placed on carbohydrate and protein ($p= .463$).

One-way ANOVA tests indicated that there was no significant difference between the fixed breakfast cereals macronutrient β coefficients, $F(2, 168)=0.122$ $p= 0.885$ or the variable breakfast cereals macronutrient β coefficients $F(2, 171)=0.283$, $p= .754$. This suggests that participants did not place a different amount of value on each macronutrient when choosing between the breakfast cereals.

Are participant's macronutrient valuations similar at lunchtime and at breakfast?

The macronutrient β coefficients were correlated to determine whether participant's macronutrient valuations were similar in both the lunchtime foods and the breakfast cereals. In the variable portion size condition, there was no significant correlation between the lunchtime foods and breakfast foods, mean β coefficients for protein ($r= 0.081$, $p= 0.544$), carbohydrate ($r= -0.071$, $p= 0.599$) or fat ($r= -0.042$, $p= 0.756$).

In the fixed portion size condition, the Pearson correlation indicated that there was not a significant relationship between the breakfast cereal macronutrient valuations and lunchtime foods macronutrient valuations. Weak relationships were observed for protein (r= 0.033, p= 0.808), carbohydrate (r= 0.002, p= 0.986) and fat (r= -0.090, p= 0.509). This suggests that the value that a person places on protein, carbohydrate and fat at lunchtime does not correspond to the same value at breakfast. In other words, there is little evidence that patterns of valuation generalize across meals.

3.3.5 Do participants in the fixed and variable portion size condition value macronutrients equally?

Three separate, independent sample t-tests were conducted to determine whether macronutrient valuation differed between the fixed and variable portion size conditions. Only the lunchtime β coefficients were compared in this analysis. Protein valuation was not significantly different between the fixed (M= 0.0172, SE= 0.0028) and variable (M= 0.0110, SE= 0.0019) portion size condition $t(100.25)$= -1.82, p= 0.0715. This indicates that individuals in the fixed and variable portion size condition placed a similar amount of valued on protein.

There was a significant difference in the carbohydrate β coefficients in the fixed portion size condition (M= 0.0130, SE= 0.0016) and the variable (M= 0.0085, SE= 0.0015) portion size condition, $t(112.1)$= -2.05, p= 0.04. There was also a significant difference in the fat β coefficients in the fixed (M= 0.0003, SE= 0.0008) and the variable (M= -0.0018, SE= 0.0005) portion size condition, $t(102.09)$= -2.21, p= 0.03. These results indicate that overall participants in the fixed portion size condition valued carbohydrate and fat significantly more than participants in the variable portion size condition.

3.3.6 Exploratory: Does perceived social status influence macronutrient valuation?

Figure 3-2 displays the significant, positive relationship between participant's carbohydrate valuations and their perceived social status scores (r= -0.410, p < 0.05). There was not a significant relationship between participant's perceived social status scores and their protein valuations (r= -0.235, p= 0.179) or their fat valuations. (r= 0.204, p= 0.246).

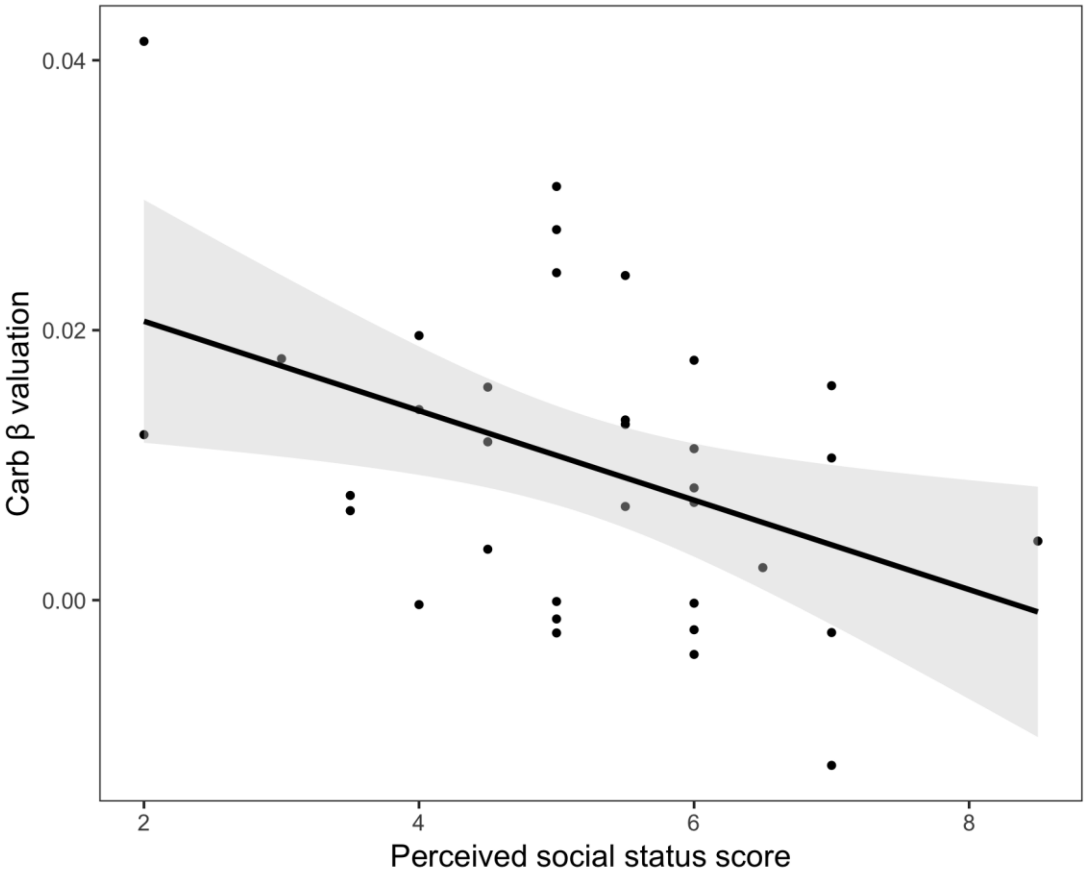

Figure 3-2 Relationship between participants' β coefficients for carbohydrate and their perceived social status score (shaded regions 95% CI)

Table 3-7 displays the β coefficients and standard error for the multilevel binary logistic regression model investigating whether perceived social status influences macronutrient valuation when controlling for participant's socioeconomic status (IMD quintile scores).

Table 3-7 *Summary of multilevel binary logistic regression analysis variables predicting food choice, with interaction terms between each predictor variable.*

	β	SE
Intercept	0.1582	0.2542
Protein difference (kcal)	-0.0021	0.0053
Carbohydrate difference (kcal)	0.0249 ***	0.0045
Fat difference (kcal)	-0.0004	0.0026
Perceived social status	-0.0205	0.0309
IMD Quintile score	-0.0018	0.0559
Protein* Perceived social status	0.0006	0.0006
Carbohydrate* Perceived social status	-0.0020 ***	0.0005
Fat* Perceived social status	0.0007	0.0003
Protein* IMD Quintile score	0.0022	0.0012
Carbohydrate* IMD Quintile score	-0.0015	0.0009
Fat* IMD Quintile score	-0.0012	0.0006

*Note: *** p< .001, ** p< .01, * p< .05; IMD Quintile scores were used as a control variable*

Figure 3-3 shows the significant interaction between perceived social status and carbohydrate difference ($p < .001$).

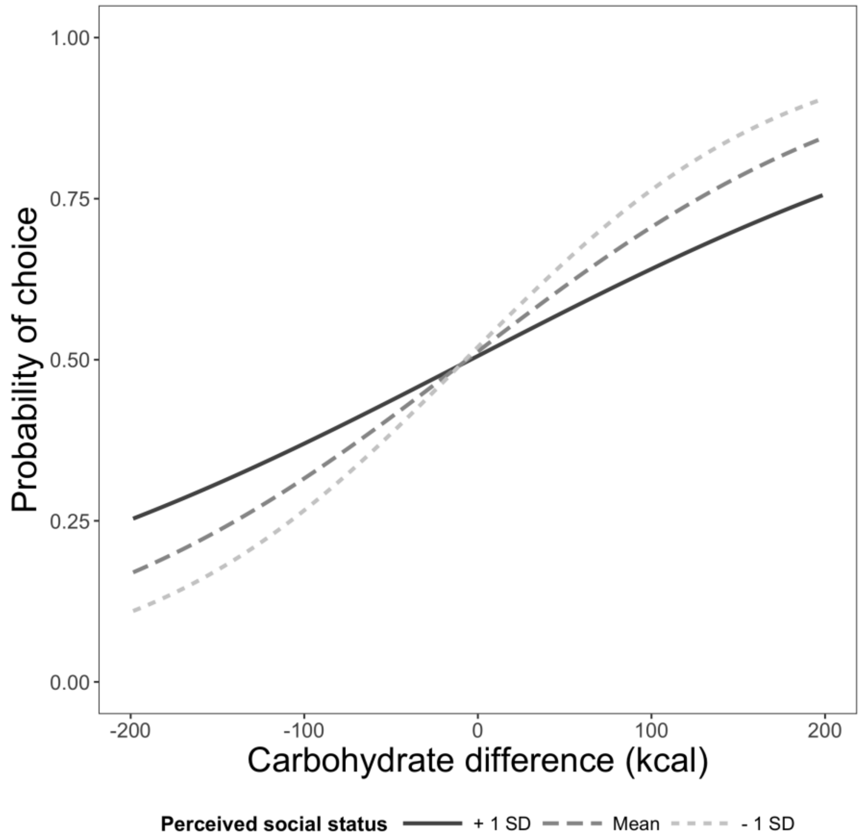

Figure 3-3 *The interaction between the probability of choosing a food as the carbohydrate difference (kcal) increases shown for low (-SD), average (mean) and high (+SD) perceived social status*

Calorie for calorie, carbohydrate was a stronger predictor of choice in participants who perceived themselves to have low social status. In other words, participants with lower perceived social status value and select foods with a higher carbohydrate content more often than participants with higher perceived social status. There was not a significant interaction between perceived social status and fat difference ($p = 0.061$) or protein difference (p= 0.379). This suggests that an individual's perceived social status does not influence their protein and fat valuations.

The interaction between perceived social status and carbohydrate valuation was significant after controlling for individual's socioeconomic status (IMD). This indicates that perceived social status contributes towards macronutrient valuation independent of an individual's actual socioeconomic status. The results also indicate that socioeconomic status, measured using IMD scores, did not interact with any of the macronutrients. This suggests that an individual's socioeconomic status has no influence on their macronutrient valuations.

3.4 Discussion

The results of the present study indicate that the macronutrient valuation hierarchy for lunchtime foods remained the same as previously seen in Chapter 2. Calorie for calorie, protein and carbohydrate were valued significantly more than fat. This order of value was the same in both the fixed and the variable portion size condition. This adds support to previous results in this thesis, specifically the macronutrient valuation hierarchy and contributes towards the reliability of the binary forced-choice task as a measure of macronutrient valuation.

The results indicated that participant's macronutrient valuations for the lunchtime foods did not correspond with their macronutrient valuations for breakfast cereals. This suggests that people might have different valuations for different meals. As previously discussed, carbohydrate intake is greater at breakfast-time and decreases throughout the day, whereas protein and fat intake increases at lunchtime and dinnertime (de Castro, 1987). The satiating value of foods decrease throughout the day (de Castro, 2004) and therefore protein might be valued more later in the day, at lunchtime or dinnertime, because is the most satiating macronutrient. Socioeconomic differences also play an important role in determining food intake at different mealtimes. Höglund, Samuelson and Mark (1998) found that children with higher socioeconomic status were more likely to eat a healthy, high-fiber breakfast and children with lower socioeconomic status were more likely to skip breakfast.

Due to some methodological issues, further research is needed to investigate whether macronutrient value is consistent across different food groups and meals.
The range in carbohydrate, protein and fat content across all of the breakfast cereals is relatively small, compared to the macronutrient composition range in the lunch times foods see Table 3-8 for comparison). This might explain why there was no difference in the value placed on protein, carbohydrate and fat at breakfast.

Table 3-8 *The macronutrient composition range and difference (g/100g) in the lunchtime and breakfast foods*

	Lunchtime range (g/100g)	Lunchtime difference (g)	Breakfast range (g/100g)	Breakfast difference (g)
Protein	0.5 - 27	26.5	4.5 – 12	7.5
Carbohydrate	0.5 - 48.9	48.4	63 – 87	24
Fat	0.5 - 22.2	21.7	0.6 - 17	16.4

Also, carbohydrate was the largest percentage of energy in the breakfast cereals, whereas the lunchtime foods included a range of high-carbohydrate, high-protein and high-fat foods. In some individuals, the underlying mechanism used to differentiate the macronutrient content of food may not be as sensitive to small differences. Future research investigating macronutrient valuation in breakfast foods should include breakfast foods with a wider range of macronutrient contents, such as eggs and bacon that have a low-carbohydrate/ high-protein content.

There was considerable variability in macronutrient value between participants in the breakfast cereals, especially for protein valuation. One explanation might be that several of the breakfast cereals were unfamiliar to the participants. Only five out of fifty-seven participants in the fixed condition and eight out of fifty-eight participants in the variable condition were familiar with all of the breakfast cereals. Familiarity is an important factor to consider when evaluating food choices. Learned sensory-characteristics of foods can signal the macronutrient content of food but if the food is unfamiliar then these sensory-characteristics are unknown (Mccrickerd & Forde, 2016). For example, sweet tastes can signal carbohydrate content and savoury tastes can signal protein content (Van Dongen et al., 2012). However, if someone has never eaten a food before (they are unfamiliar with the food) then they will not have experienced the taste of that food and ultimately predicting the macronutrient content from taste signals will be more difficult. Therefore, when exploring the relative value that individual's place on each macronutrient it is important to consider their familiarity with the test foods. A pilot study would have been beneficial to measure the familiarity of the breakfast cereals included in the study.

There are some subtle differences in macronutrient valuation for the lunchtime foods between the fixed and variable portion size condition. Participants in the fixed portion size condition valued fat and carbohydrate, calorie for calorie, more than people in the variable portion size condition. The relative value placed on protein difference did not differ between the two portion size conditions. Participants in the fixed and variable portion size condition valued protein to a similar extent. Despite these differences the macronutrient valuation hierarchy protein > carbohydrate > fat was demonstrated in both the fixed and variable portion size conditions. Therefore, it can be concluded that when measuring macronutrient valuation portion size is not a considerable factor. Although, future studies may decide to include variable portion sizes, opposed to fixed portion sizes, in the task to reduce the possibility that participants are selecting the food based on the amount of food on the plate.

The exploratory analysis results suggest that perceived social status influences carbohydrate valuation independent of socioeconomic status. Participants with lower perceived social status valued carbohydrates more than participants with higher perceived social status. There was no effect of perceived social status on the relative value placed on fat or protein. Previous research suggests that people who had lower perceived social status were more vulnerable to increased feelings of stress and depression (Shea, 2014). This could help explain why individuals who perceive themselves to have lower social status value carbohydrates to a greater extent. Some individual's increase their preference and intake of carbohydrate-rich food in order to alleviate negative feelings (Gibson, 2012). It would be interesting to add a measure to determine whether participants are considered 'carbohydrate cravers' (Wurtman & Wurtman, 1995).

In this study, participants were asked to write down their postcodes after completing all of the computer tasks. Unfortunately, this resulted in either incorrect or unidentifiable postcodes. Therefore, the sample was reduced to 22 participants. This issue is addressed in chapter 4, where postcodes are electronically recorded.

In conclusion, there was little evidence that macronutrient valuation in one meal generalises to valuation in a different meal. However, at this stage potential methodological problems might account for this and so this hypothesis cannot be ruled out with certainty. The results from this study also highlight the individual differences that can influence macronutrient valuation. The association between perceived social status and macronutrient valuation is further discussed in Chapter 4.

Chapter 4 Investigating the influence of perceived social status on macronutrient valuation

4.1 Introduction

As discussed in Chapter 1, socioeconomic status and perceived social status are key factors influencing food intake and weight gain. To reiterate, socioeconomic status is the social position of an individual in society and is measured using social and economic variables such as an individual's household income, occupation and education (Adler et al., 1994). Perceived social status is an individual's interpretation of their own standing in society in relation to others, often represented as a social hierarchy (Adler et al., 2000). The relationship between obesity and socioeconomic status has been extensively researched and results indicate that in developed nations, low levels of socioeconomic status are associated with increased food intake and subsequently, increased BMI (Baum & Ruhm, 2009).

Financial instability is a marker for low socioeconomic status and could lead people to feel food insecurity; defined as "limited or uncertain ability to acquire nutritionally adequate and safe food in socially acceptable ways" (Anderson, 1990, as cited in Castillo et al., 2012). The insurance hypothesis (Nettle et al., 2017) provides an evolutionary explanation for the association between food intake and socioeconomic status. It suggests that humans use environmental cues, that signal when food availability is uncertain, and adjust their energy intake to exceed levels of energy expenditure. Adults with low socioeconomic status may interpret food insecurity as a cue to increase fat stores, which can buffer the risk of starvation until food resources are replenished. Nettle et al., (2017) conducted a meta-analysis and found that food-insecure participants were 21% more likely to be overweight than food-secure participants.

Several meta-analyses have been conducted over the last decade, investigating the association between socioeconomic status and obesity (McLaren, 2007; Nettle et al., 2017; Sobal & Stunkard, 1989). The overall finding from these studies is that the association between obesity and socioeconomic status is complex and it varies across gender and country. The relationship between increased food insecurity and increased body weight is stronger for women than men (Nettle et al., 2017). This difference between genders could be because of women's need to support a pregnancy. The risk of losing fat stores would risk their fertility and the child's health.

The meta-analysis results (Sobal & Stunkard, 1989) also found that the association between obesity and low socioeconomic status was only seen in high-income countries and not seen in low-income countries. This could be because high-energy dense foods are more available in high-income countries (Drewnowski & Popkin, 2009) and thus when food-insecure adults do have access to food, it is often high-calorie, processed foods. Furthermore, energy dense, processed foods are usually cheaper (Drewnowski & Specter, 2004) and are therefore more accessible to people who are finically insecure. People in low-income countries often have restricted access to energy-dense foods (Wetsman & Marlowe, 1999) and therefore, struggled to gain and maintain body weight. For example, Gulliford, Nunes and Rocke (2006) found that in Trinidad and Tobago, a low-income country, food-insecure people did not have higher BMIs than food-secure people. The inconsistent findings across gender and high- / low- income countries suggest that the insurance hypothesis alone cannot sufficiently explain the relationship between socioeconomic status and obesity and it is important to consider other mediating factors such as wellbeing and environment.

There are several studies that support the relationship between low socioeconomic status and increased weight gain (McLaren, 2007). However, interventions designed to increase fruit and vegetable consumption and reduce financial burden can increase caloric intake and increase body weight (Leroy, Gadsden, Gonzalez de Cossio, & Gertler, 2013). This is the opposite pattern of the original intention. Also, there are individual's living in deprived areas with low socioeconomic status that are not overweight and therefore, other factors need to be considered when evaluating and planning interventions to reduce the risk of developing obesity.

Recently, the relationship between perceived social status and food choice has been explored (Cardel et al., 2016; Cheon et al., 2018). Perceived social status captures an individual's evaluation of their own assets (wealth, respect and opportunities for social mobility) compared to others (Singh-Manoux, Adler, & Marmot, 2003). Often an individual's perceived social status is highly correlated with their socioeconomic status (Demakakos, Nazroo, Breeze, & Marmot, 2008). Despite a strong relationship between socioeconomic status and perceived social status, Singh-Manoux, Marmot and Adler (2005) found that perceived social status was a stronger predictor of obesity than socioeconomic indicators. This highlights the importance of considering both socioeconomic status and perceived social status when evaluating the effects on obesity and food intake. Low socioeconomic status does not necessarily mean low perceived social status and vice versa. For example, individuals with low socioeconomic status may perceive themselves to have high social standing within their family, social group or religion.

Research investigating the relationship between perceived social status and obesity suggests that people who perceived themselves to be at the lower end of the social hierarchy are more likely to be overweight (Adler et al., 2000). There is also evidence to suggest that experimentally manipulating the experience of low perceived social status is sufficient enough to increase participant's preference and intake of energy dense foods (Cheon & Hong, 2017). The relationship between perceived social status and increased caloric intake might have served as an adaptive function, developed to increase an individual's chances of survival (Cheon, Lim, McCrickerd, Zaihan, & Forde, 2018). For example, among social animals, social standing within the group is often related to greater access to food and mates (Arce, Michopoulos, Shepard, Ha, & Wilson, 2010). Therefore, animals with lower social standing must increase their food intake, when food is available, and select energy dense foods, in order to avoid starvation. Social standing is also an important non-food resource within human society. Financial and material resources allow humans access to food and if an individual believes that their social and economic resources are scare or deprived, then they may increase their preference and intake of energy dense foods (Briers & Laporte, 2013). These results suggest that perceived social status is an important factor contributing towards the rise in obesity and interventions should focus on psychological factors that address feelings of resource deprivation.

An additional factor that should be considered when evaluating the relationship between perceived social status and food intake is an individual's mood and wellbeing. Evidence suggests that negative affect moderates the relationship between socioeconomic status and health (Operario, Adler, & Williams, 2004). Psychological pathways such as depression and stress are associated with low levels of social status (Baum, Garofalo, & Yali, 1999; Miech & Shanahan, 2000) and the physiological responses to depression and stress could in turn influence food and calorie intake, as discussed in Chapter 1.

As seen in Chapters 2 and 3, a binary forced-choice task was developed to measure individual macronutrient valuations. In this experiment, the binary forced-choice task was used to gain further insight in to the relationship between macronutrient valuation and perceived social status. The aim was to determine whether individuals with lower perceived social status indicate a specific tendency to value protein, carbohydrate and fat differently to those with a higher perceived social status.

4.2 Method

4.2.1 Design

Participants were asked to attend two test sessions, exactly one week apart. The two test sessions were identical in procedure. To reiterate, the data collected for this experiment is the same data set used in Chapter 2, Part 2.

4.2.2 Participants

Based on a medium effect size (Cohen, 1992), a total of 92 participants were recruited. Of those 92, 8 participants were unable to attend their second test session and were removed from the data set. The remaining 84 participants (female= 57) had a mean age of 25.13 years (SD= 8.37, range= 19 - 71) and 73% of participants BMI scores were in the normal range (M= 23.03, SD= 4.21). Participants were recruited from the University of Bristol (UK) and from the Bristol area. All participants were reimbursed for their time with £15 for completing both test sessions and £10 for completing only the first test session. Participants were excluded if they were vegan or vegetarian and if they had any food allergies or food intolerances.

4.2.3 Food stimuli

Images of 25 foods were used in measures of food choice, expected satiety, perceived healthiness, liking and familiarity. To ensure that foods were familiar, foods that are commonly consumed in the United Kingdom were selected (Henderson et al., 2002). The foods chosen had a range of macronutrient compositions and the correlation between macronutrients was weak; protein and carbohydrate (r= -0.363, p< .001), fat and carbohydrate (r= -0.324, p< .001) and protein and fat (r= 0.316, p< .001). The macronutrient composition and the energy density of the 25 foods used in this experiment are shown in Table 2-6. Every food was photographed in 100-g portions on a white plate (255-mm diameter) using a high-resolution digital camera. The name of the food was presented in white font in the top-left-hand corner of every image.

4.2.4 Measures of macronutrient value

The binary forced-choice task used to measure macronutrient valuation and measures of expected satiety, perceived healthiness, liking and familiarity are identical to the measures used in Chapter 2, Part 2.

4.2.5 Perceived social status and socioeconomic status measures

Participant's occupation, education and income were recorded as objective measures of socioeconomic status. To assess participants income, they were asked 'What is your household income per annum (pa)?' and given 6 categories ranging from 'Less than £15,000 (pa)' to 'More than or equal to £70,000 (pa)' (Operario et al., 2004). The option 'I would rather not say' was also included so that participants did not feel pressured to disclose personal information. The open-ended question 'What is your occupation?' was answered and participants also stated their highest level of qualification.

Participant's postcodes were also recorded as an objective control measure of socioeconomic status. Postcodes were used to measure participants Index of Multiple Deprivation (IMD) (NPEU, 2010). The IMD is a UK government study of deprived areas in England and each postcode is given an IMD score and scores are grouped in to IMD quintiles (1-5). The scores range from less than or equal to 8.49 representing the least deprived areas and more than or equal to 34.18 representing the most deprived areas. The IMD quintile groups range from 1 – 5; 1 represents the least deprived area and 5 represents the most deprived area. The MacArthur Scale of Subjective Social Status (Adler et al., 2000) was used in this study to measure participant's perceived social status. This measure is described, in detail, in Chapter 3.

4.2.6 Questionnaires

A pre-session questionnaire was completed using the Bristol Online Survey to measure participant's age, gender and preferred test-session timeslot.

The Dutch Eating Behaviour Questionnaire (van Strien et al., 1986) was used to measure participant's dietary eating behaviour. A full description of this questionnaire is detailed in Chapter 2, Part 2.

The Positive and Negative affect scale (PANAS) (Watson, Clark, & Tellegen, 1988) is a self-report questionnaire that includes 20-items, measuring an individual's positive and negative affect. Participants are presented with an alternating list of 10 positive affect words and 10 negative affect words and asked to 'Indicate to what extent you felt this in the past week.' Each item is rated on a 5-point scale from 'Very slightly or not at all' (1) to 'Extremely' (5). For each individual, a positive affect score and a negative affect was derived by summing the numerical rating for each positive and negative item. Scores can range from 10-50 and the higher the score the higher the level of positive or negative affect.

The Depression, Anxiety and Stress scale (DASS) (Lovibond, 1995) is a self-report measure of an individual's emotional state of depression, anxiety and stress. The 21-item questionnaire used in this study is a shortened version of the original 42-item questionnaire. Each statement on the scale is rated between 0 - 'Did not apply to me at all' and 3 - 'Applied to me very much or most of the time'. Participants are reminded that there are no right or wrong answers. Each of the three subscales contains 7-items and are scored separately, resulting in a depression, anxiety and stress score. For the final score of the 21-item DASS, the scores are multiplied by two. Each subscale is ranked either normal, mild, moderate, severe or extremely severe.

4.2.7 Procedure

Participants attended two test sessions, one week apart at exactly the same time of day. A pre-session questionnaire was completed online before attending the test session to record participant demographics. On arrival at the laboratory, each participant read through an information sheet and signed a consent form before beginning. A consent form was completed in both test sessions. The binary forced-choice task was completed first, followed by measures of expected satiety, perceived healthiness, familiarity and liking. The MacArthur Scale of Subjective Social Status, postcodes, the PANAS, the DASS and the DEBQ were then completed using the Bristol Online Survey website. Finally, height and weight were recorded. Participants were given a debrief at the end of the second test session and were reimbursed £15 cash for their time. All measures were completed in the first and second test session. The study protocol including hypotheses was preregistered with the Open Science Framework titled "Investigating individual's macronutrient valuation and whether this is influenced by perceived wellbeing." (https://osf.io/xhn9j/)

4.2.8 Statistical Analysis

Statistical power was calculated for a sample of 85 participants, based on a medium effect size (Cohen, 1992). All analyses were preformed using R software (R Core Team, 2017) and the significance value was set at $p < .05$. The binary logistic regression models were completed using the lme4 add-on package (Bates et al, 2015) and figures were created using the ggplot2 add-on package (Ginestet, 2011).

Participant's perceived healthiness, liking and expected satiety scores were averaged to identify normality. The percentage of familiar foods was checked to ensure that participants were familiar with the food stimuli being chosen. The range, mean and standard deviation scores were calculated for participant's socioeconomic measures to check for a normal distribution across all categories. Before analysis, the protein, carbohydrate and fat content per/100-g was multiplied by 4, 4, and 9, respectively to calculate the protein, carbohydrate and fat content in kcal/ per 100-g. For each binary forced-choice trial, a separate 'difference score' was calculated (right-hand food – left-hand food) for protein, carbohydrate and fat.

Multilevel modeling was used to assess how perceived social status interacts with the macronutrient content of the food to predict food choice. Each participant completed 600 trials, 300 trials over two test sessions, where each food was compared to all other foods. Therefore, the assumption of independence of ratings is violated. To account for participant variability, trials were nested within participants in a multilevel binary logistic regression model. In each model, every β coefficient refers to the odds of choosing the right-hand food when the right –hand food contains 1kcal/ 100g more protein than the left-hand food. These β coefficients quantify macronutrient valuation. The β coefficients for carbohydrate and fat can be interpreted in the same way.

Model 1 – Does perceived social status influenced macronutrient valuation?

Model 1 was a multilevel binary logistic regression that included interaction terms to investigate whether participant's perceived social status influenced their macronutrient value. The outcome variable was food choice and there were three interaction terms; protein difference score, carbohydrate difference score and fat difference score each separately with perceived social status score. The interaction terms showed whether calorie for calorie, protein, carbohydrate or fat were stronger predictors of food choice for people with lower or higher levels of perceived social status.

Model 2 – Does perceived social status influence macronutrient valuation, independent of socioeconomic status (IMD)?

Model 2 was run to investigate whether perceived social status influenced macronutrient value when controlling for participant's socioeconomic status (IMD). The IMD quintile groups were reversed so that 1 represented a low socioeconomic status and 5 represented a high socioeconomic status. This was to ensure that both IMD quintile scores and perceived social status scores were both in the same direction. The outcome variable was food choice and there were six interaction terms entered in to the model; protein difference score, carbohydrate difference score and fat difference score, each separately with perceived social status score and IMD quintiles. If the interaction terms remain significant between each macronutrient and perceived social status, then this indicates that perceived social status influences macronutrient value even when controlling for an individual's socioeconomic status.

Is perceived social status influenced by participant's socioeconomic status, emotional state of depression, stress and anxiety and their positive and negative affect?

A multiple regression model was run to determine the amount of variance in participant's perceived social status scores, that is explained by participant's socioeconomic status (IMD), emotional state of depression, stress and anxiety and their positive and negative affect. Participant's socioeconomic status, levels of depression, anxiety and stress and their positive and negative affect were assessed using difference measures and therefore the DASS, PANAS and IMD quintile scores were standardised to z scores. The multiple regression model outcome variable was perceived social status scores and the predictor variables were participant's scores for socioeconomic status, depression, anxiety, stress and their level of positive and negative affect.

Exploratory analysis – Does perceived social status influence individual's value for energy density?

An exploratory analysis was conducted to investigate the influence of perceived social status on individual's valuation of energy density. For each participant and each binary forced-choice trial, a separate 'energy-density difference score' was calculated. The energy density (kcal/g) of the left-hand food was subtracted from the energy density of the right-hand food. A multilevel binary logistic regression model was run with food choice as the outcome variable and the predictor variable was energy-density difference scores. Two interaction terms were entered (1. between perceived social status and energy density difference scores and (2. between socioeconomic status (IMD) and energy-density difference scores. This model indicated whether energy density is a stronger predictor of food choice in people with high or low perceived social status and in people with high or low socioeconomic status.

4.3 Results

4.3.1 Participant demographics

Participant demographics are summarised in Table 4-1. Values include gender, age, hunger ratings, BMI and the three DEBQ subscales; emotional eating, restraint and external eating.

Table 4-1 *Participant's demographics*

	M	SD	Range
Gender (% female)	68		
Age (years)	25.1	8.4	19 - 71
BMI	23.0	4.2	14.7 – 39.7
Hunger (mm)	5.6	1.8	0 – 9
DEBQ Emotional Eating	2.4	0.8	1.0 – 4.6
DEBQ Restraint	2.4	0.7	1.0 – 3.9
DEBQ External Eating	3.3	0.6	1.9 – 4.8

4.3.2 Perceived social status and socioeconomic measures

The majority of participants (96%) had continued their education post-16 and over half (63%) had completed a university undergraduate degree. Regarding participant occupation, 62% were students and employment was split between full-time (38%), part-time (24%) and unemployed (38%). Table 4-2 shows the means and standard deviations for participant's index of multiple deprivation (IMD) scores and quintile groups and their perceived social status scores (averaged across both test sessions). The IMD tool used to convert postcodes to IMD scores only accepted English postcodes and therefore a total of 11 postcodes were excluded.

Table *4-3* shows the percentage of participants in each household income (per annum) category.

Table 4-2 *Participant's mean (M) and standard deviation (SD) scores for IMD, IMD quintile and perceived social status*

Measure	Range	M	SD
IMD score	2.6 – 46.7	13.9	9.4
IMD Quintile (1 – 5)	1 – 5	2.3	1.2
Perceived Social status (10 ladder rungs)	2 – 8	5.6	1.4

Table 4-3 *Percentage of participant's in each household income (pa) category*

Household Income (pa)	% Participants
<= £15,000	9
= £15,000 < £26,000	22
= £26,000 < £35,000	12
= £35,000 < £50,000	13
= £50,000 < £70,000	18
>- £70,000	9
"I would rather not say"	17

4.3.3 Does perceived social status influence macronutrient value?

Table 4-4 displays the β coefficients and standard error values for model 1 and model 2. Model 1 investigated the influence of perceived social status on macronutrient valuation and model 2 investigated the influence of perceived social status on macronutrient valuation controlling for participant's socioeconomic status. Figure 4-1 displays the interaction terms in model 1.

Table 4-4 *Summary of Model 1 and Model 2 multilevel binary logistic regression variables predicting food choice*

*Note: *** p< .001, ** p< .01, * p< 05*

	Model 1 – perceived social status only		Model 2 – perceived social status and IMD	
	β	SE	β	SE
Intercept	0.0989 *	0.0471	0.1931	0.0699
Protein difference (kcal)	0.0094 ***	0.0008	0.0113 ***	0.0012
Carbohydrate difference (kcal)	0.0122 ***	0.0006	0.0014 **	0.0008
Fat difference (kcal)	0.0009 **	0.0004	-0.0012 *	0.0005
Perceived social status	-0.0194 *	0.008	-0.0222	0.0085
Protein* perceived social status	-0.0008 ***	0.0001	-0.0011 ***	0.0001
Carbohydrate* perceived social status	-0.0015 ***	0.0001	-0.0014 ***	0.0001
Fat* perceived social status	-0.0004 ***	0.0001	-0.0003 ***	0.0001
Index of multiple deprivation (IMD) quintile score			-0.0205	0.0114
Protein* IMD quintile score			0.0001	0.0002
Carbohydrate* IMD quintile score			0.0028 ***	0.0001
Fat* IMD quintile score			0.0004 ***	0.0001

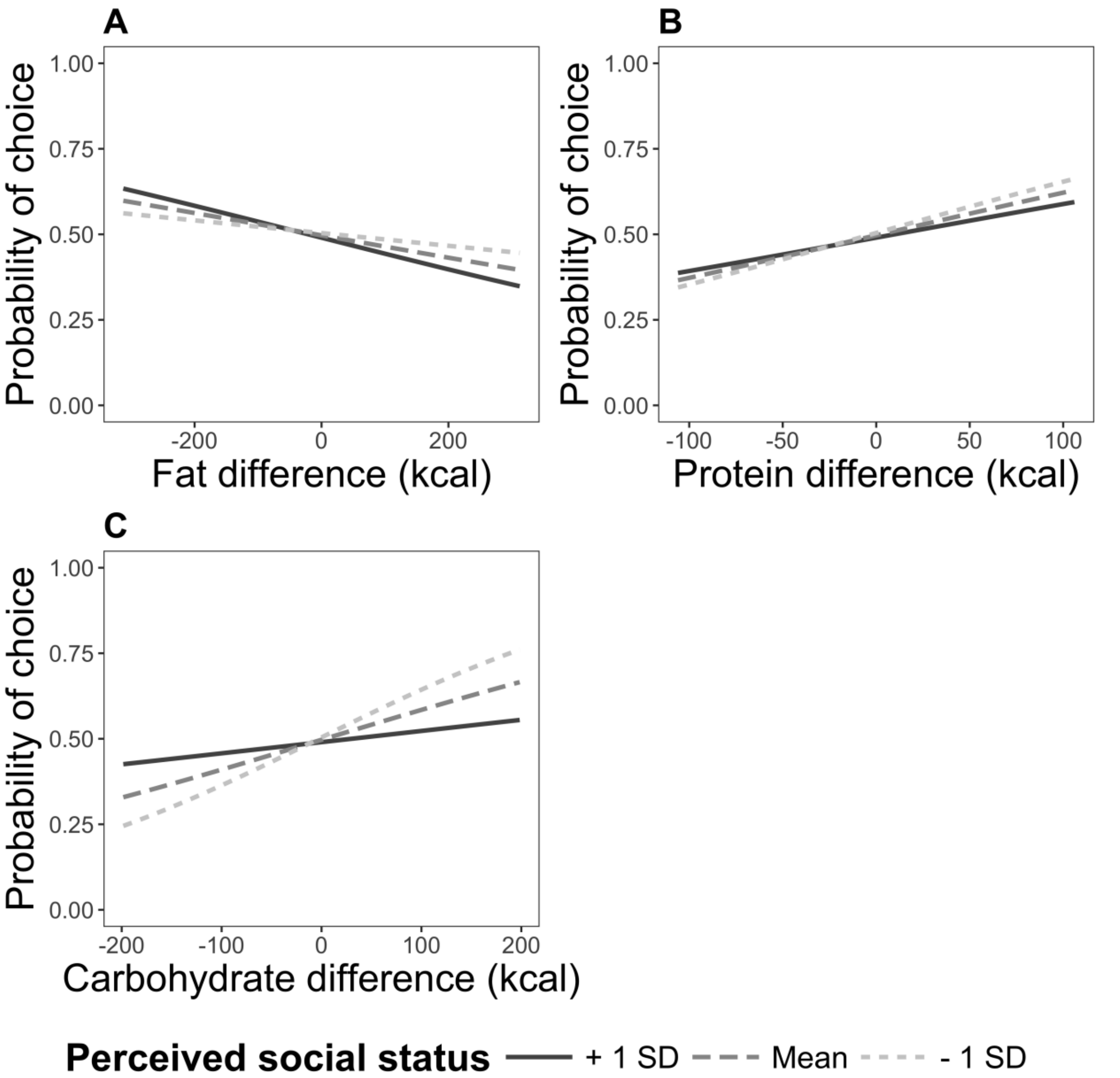

Figure 4-1 *The interaction between the probability of choosing a food as the A) fat difference (kcal), B) protein difference (kcal) and C) carbohydrate difference (kcal) increases - shown for low (-SD), average (mean) and high (+SD) perceived social status*

Model 1 indicates that there is a significant negative interaction between perceived social status and all three macronutrients. This suggests that calorie for calorie, protein, carbohydrate and fat are stronger predictors of choice for people with lower perceived social status. In other words, people with lower perceived social status value protein, carbohydrate and fat to a greater extent than people with higher perceived social status.

The strongest interaction effect was the relationship between carbohydrate difference and perceived social status.

Model 2 also shows a significant negative interaction between perceived social status and all three macronutrients, therefore suggesting that the relationship between perceived social status and macronutrient valuation is independent of participant's socioeconomic status. Additionally, model 2 shows a significant positive interaction between socioeconomic status and carbohydrate difference and socioeconomic status and fat difference. This indicates that carbohydrate difference and fat difference are stronger predictors of choice for people with higher socioeconomic status. In other words, people with higher socioeconomic status value carbohydrate and fat more than people with lower socioeconomic status. There is not a significant interaction between socioeconomic status and protein difference suggesting that socioeconomic status does not influenced protein valuations.

4.3.4 Is perceived social status influenced by an individual's socioeconomic status and wellbeing?

A multiple linear regression analysis was run to determine the extent to which an individual's socioeconomic status and wellbeing contributed towards their perceived social status. Wellbeing was assessed by participant's emotional state of depression, stress, anxiety and positive and negative affect and their IMD quintile scores represented their socioeconomic status. The outcome variable in the multiple linear regression analysis was perceived social status scores and the predictor variables were participant's emotional state of depression, stress, anxiety, positive and negative affect and their IMD quintile scores. The results indicate that the predictors explained 2% of the variance in perceived social status (adjusted $R^2 = 0.021$, $F(6, 66) = 1.259$, $p = 0.288$). Participant's socioeconomic status and wellbeing did not significantly predict their perceived social status scores; IMD quintile ($\beta = -0.184$, $p = 0.332$), depression ($\beta = -0.454$, $p = 0.125$), stress ($\beta = -0.276$, $p = 0.357$), anxiety ($\beta = 0.085$, $p = 0.741$), positive affect ($\beta = -0.270$, $p = 0.208$) and negative affect ($\beta = 0.387$, $p = 0.105$). This suggests that an individual's socioeconomic status and their perceived social status are separate measures and supports the idea that an individual could have high socioeconomic status and low perceived social status and vice versa. The results also suggest that participant's perceived social status was not influenced by their wellbeing.

4.3.5 Exploratory analysis

Table 4-5 includes the β coefficients and standard error values for the multilevel binary logistic regression model investigating whether perceived social status influenced participant's valuation of energy density, independent of socioeconomic status. *Figure 4-2* shows the interaction terms from *Table 4-5*.

Table 4-5 *Summary of the multilevel binary logistic regression analysis variables predicting food choice including interaction terms between each variable*

	β	SE
Intercept	0.1985	0.0684
Energy density difference (kcal)	0.1428 ***	0.0428
Index of multiple deprivation (IMD)	-0.0225	0.0111
Perceived social status	-0.0224	0.0083
Energy density * IMD	0.0568 *	0.0067
Energy density * Perceived social status	-0.0544 ***	0.0054

*Note: *** $p< .001$, ** $p< .01$, * $p< .05$*

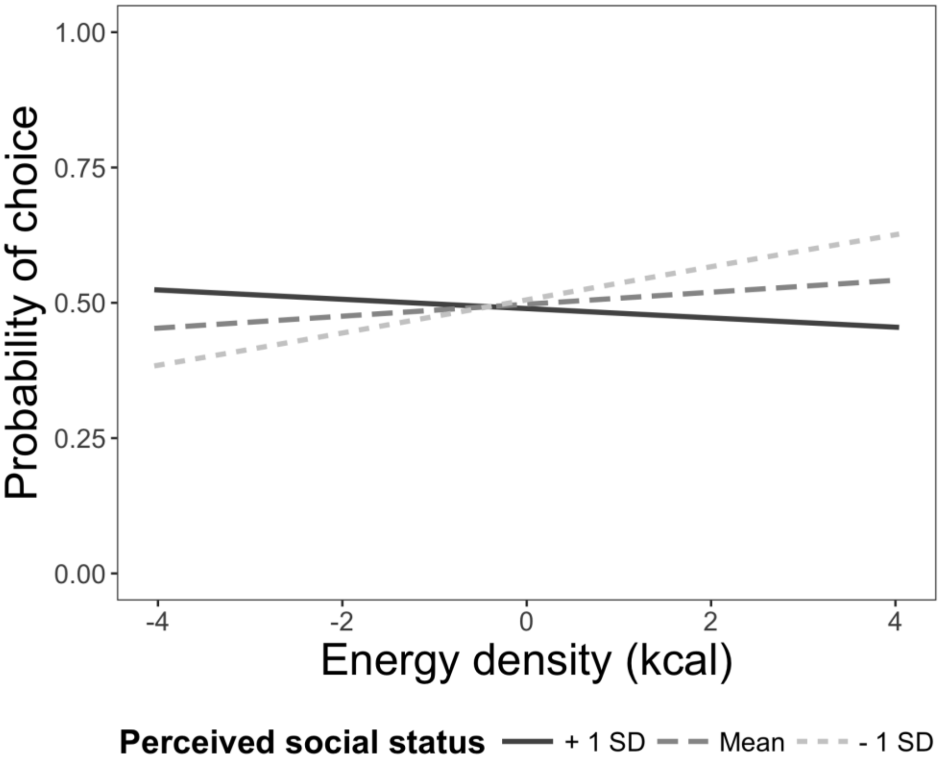

Figure 4-2 *The interaction between the probability of choosing a food as the difference in energy density (kcal) increases - shown for low (-SD), average (mean) and high (+SD) perceived social status*

Figure 4-2 indicates that there was a significant interaction between energy density difference and perceived social status. Energy density predicted food choice to a greater extent for people with low perceived social status. This suggests that people with lower perceived social status value energy dense foods more than people with higher perceived social status. These results are significant after controlling for participant's socioeconomic status (IMD). In order to probe the interaction effect between perceived social status and energy density the Johnson-Neyman technique was used (Johnson & Fay, 1950). Table 4-6 summaries the findings of the Johnson-Neyman technique.

Table 4-6 *Summary of the Johnson Neyman technique to probe the interaction between perceived social status and energy density as predictors of food choice*

Value of perceived social status	Slope of energy density
7.08 (+1 SD)	-0.03
5.64 (Mean)	0.04 *
4.19 (-1 SD)	0.12*

*Note: * p< .05*

The Johnson-Neyman technique identified that when participant's perceived social status score was below 7.08, energy density significantly predicts food choice. When a participant's perceived social status score was above 7.08, the interaction was non-significant. This therefore suggests that for people with higher perceived social status (a score of 7.08 – 10), the value they place on energy density does not influence their food choices.

4.4 Discussion

The results from this study demonstrate that individual differences, specifically perceived social status, can influence macronutrient valuation. As predicted, the relative value placed on protein, carbohydrate and fat, calorie for calorie, varied across levels of perceived social status. People with lower perceived social status valued protein, carbohydrate and fat to a greater extent than people with higher perceived social status. The results from the exploratory analysis also suggest that perceived social status influences the amount of value individual's place on energy density. A larger difference in energy density was a stronger predictor of food choice for people with lower perceived social status, suggesting that they valued foods with higher energy densities more than people with higher perceived social status.

This supports previous research that found that the mere experience of low perceived social status could stimulate appetite and caloric intake (Cheon & Hong, 2017). It is also consistent with research conducted by Cheon, Lim, McCrickerd, Zaihan and Forde (2018) who found that participants in the low subjective social status condition could perceptually discriminate the energy density of beverages. The ability to identify the macronutrient composition and energy density of foods may have served our hunter-gather ancestors as an adaptive function for the successful maintenance of energy and nutrient balance (Brunstrom & Cheon, 2018). Hunters would have wanted to secure nutrient and energy dense foods in order for the cost involved in obtaining and consuming food to be beneficial.

Socioeconomic resources can secure access to food and, if an individual perceives these resources to be insecure this could translate to feelings of food insecurity (Sim, Lim, Forde, & Cheon, 2018). Historically, humans lived in large groups to ensure that members could share the responsibly of foraging for food (Brunstrom & Cheon, 2018). These social resources were an important source of food security and therefore, humans might have developed the ability to identify social rank. Members who perceived themselves to have a low social rank might have increased their food intake to reduce the risk of starvation if ostracized (Sproesser, Schupp, &

Renner, 2014). Although the risk of being ostracized is still relevant, in the modern environment it is unlikely to result in starvation. Due to the industrialized food environment, people do not need to rely on their social group to provide them with food.

There are examples in the modern eating environment that demonstrate the relationship between feelings of insecurity and increased energy intake. Cardel et al (2016) used a rigged version of the board game "Monopoly", that differed in degrees of 'privilege', to manipulate participant's feelings of high or low social status. During an ad-libitum meal, after the game, participants in the low social status condition consumed 130 calories more, relative to the high social status condition. They also consumed a greater amount of foods that contained high-fat and high-sodium levels. Similarly, football supporters in the USA increase their caloric intake after their football team lose a match (Cornil & Chandon, 2013). Football supporters often perceive their team's failures are their own (Hirt, Zillmann, Erickson, & Kennedy, 1992) and these failures could translate as feelings of perceived deprivation. This indicates the importance of the association between food choice and perceived social status.

The results from this study suggest that perceived social status influences macronutrient valuations independent of socioeconomic status. People with lower perceived social status valued protein, carbohydrate and fat to a greater extent than people with higher perceived social status, after controlling for participant's socioeconomic status. Perceived social status and socioeconomic status are often highly correlated, (Demakakos et al., 2008) suggesting that an individual with high socioeconomic status will also have high perceived social status. However, an individual's beliefs about their social and economic resources can sometimes be a more accurate representation of their social standing (Operario et al., 2004). An individual's perception of their relative social standing is an additional value of their status and demonstrates the implications that their income, education and occupation have on their life. For example, two people with university undergraduate degrees have the same objective educational attainment however; the prestigious status of the institute may influence their perceived social standing in society. Therefore, an individual's perceived resources and abilities, relative to others may give a more accurate representation of health and wellbeing. This supports the idea that perceived social status and socioeconomic status are different measures and should be evaluated separately.

The study was conducted at the University of Bristol, UK; a prestigious institution within a high-income country and therefore, the sample was highly educated and financially secure. This meant that it was unlikely that any of the participants were actually at risk of food insecurity. Despite this, the participant's socioeconomic and perceived social status measures were equally distributed. This suggests that the sample was a good representation of the population within a high-income country.

Previous research has suggested that the relationship between perceived social status and increased food intake is mediated by negative affect (Bratanova, Loughnan, Klein, Claassen, & Wood, 2016). However, this effect is not always consistent and, often depends on the measure used to manipulate perceived social status (Sim, Lim, Leow, & Cheon, 2018). Within this study, participant's wellbeing (depression, anxiety, stress, positive and negative affect) did not significantly predict their perceived social status scores. This therefore suggests that perceived social status influences macronutrient valuation independent of negative feelings.

In conclusion, the results suggest that, in general people with lower perceived social status value food more than people with higher perceived social status. Therefore, suggesting that perceived insecurity and low social standing in society might play an important role in the obesogenic environment. Future interventions designed to reduce the risk of obesity should consider individual's perceived social standing and not solely focus on absolute social and economic resources.

Chapter 5 General discussion

There is evidence to suggest that humans have the ability to discriminate the macronutrient content of foods and this enables them to maintain and regulate a nutrient balance (Berthoud et al., 2012). However, it is unclear whether individual's value for protein, carbohydrate and fat is equal and therefore, the aims of this thesis were to investigate and quantify the relative value placed on each macronutrient. Within this thesis, the value of protein, carbohydrate and fat were analysed calorie for calorie, in order to compare the macronutrients relative to one another. First, after reviewing previous measures of macronutrient preference, a novel, binary forced-choice task was developed to measure macronutrient valuation. The test-retest reliability of the binary forced-choice task was measured and the results indicated a strong correlation between participant's macronutrient valuations across two-test sessions. Then, an additional food category was added to the forced-choice task to investigate whether individual macronutrient valuations remained stable across two different meal times (breakfast and lunchtime). Finally, the binary forced-choice task was used to investigate the influence of individual differences on macronutrient valuations specifically, perceived social status.

The key findings from each experimental chapter are summarised in Table 5-1. This chapter will discuss these findings in terms of theoretical implications and possibilities for future directions.

Table 5-1 *Summary of the key findings from each experimental chapter*

Chapter	Key findings
2. Development of a novel, binary forced-choice task to measure macronutrient valuation (Part 1: Feasibility)	• Macronutrient valuation can be quantified using the novel, binary-forced choice task • On a calorie for calorie basis, protein, carbohydrate and fat are not valued equally • A macronutrient valuation hierarchy was demonstrated: protein > carbohydrate > fat • Participant's macronutrient valuations in food list 1 did not correspond with valuations in food list 2.
2. Development of a novel, binary forced-choice task to measure macronutrient valuation (Part 2: Test-retest reliability)	• Participant's protein, carbohydrate and fat valuations were highly correlated across session 1 and session 2. • The binary forced-choice task had strong test- retest reliability • The macronutrient valuation hierarchy (protein > carbohydrate > fat) seen in part 1, was replicated in part 2
3. Investigating macronutrient valuation in lunchtime foods and breakfast cereals	• The macronutrient hierarchy: protein > carbohydrate > fat was seen in the lunchtime foods • Participant's macronutrient valuations were not the same for the lunchtime foods and the breakfast cereals • Participants were unfamiliar with a lot of the breakfast cereals • The exploratory analysis suggested that carbohydrate is valued to a greater extent by people with lower perceived social status, independent of their socioeconomic status

4. Investigating the influence of perceived social status on macronutrient valuation	• Participants with lower perceived social status valued protein, carbohydrate and fat more than participants with higher perceived social status, independent of socioeconomic status. • Participants with lower perceived social status also valued energy density more than participants with higher perceived social status. • Participant’s socioeconomic status and wellbeing did not predict their perceived social status

5.1 Main findings and implications

5.1.1 On a calorie for calorie basis, are macronutrients valued equally?

Across all studies, the results indicate that calorie for calorie, protein, carbohydrate and fat are not valued equally. This adds support to previous research that suggests that humans have the ability to discriminate the macronutrient content of food (Birch, 1999). People must first be able to identify the macronutrient content of food, in order to place differing amounts of value on protein, carbohydrate and fat. The results also indicate that there is considerable variability between individuals and the amount of value placed on protein, carbohydrate and fat. This variability highlights that individuals differ in their underlying sensitivity to a food's macronutrient compositions. An individual with high carbohydrate valuation would be particularly sensitive to small differences in carbohydrate content between two foods, whereas an individual with low carbohydrate valuation might not differentiate the carbohydrate content of the two foods. This could help explain why individuals differ in their food choices.

5.1.2 Are individual's macronutrient valuations consistent across different meals?

In chapter 3, the value that a person placed on protein, carbohydrate and fat at lunchtime did not correspond to the same values at breakfast. A possible explanation for this is that different macronutrients are required and prioritised at different times of day. Previous research indicates that at breakfast, our carbohydrate intake is high and protein intake is low and protein intake is higher at lunch-time and dinner and carbohydrate intake is lower (de Castro, 1987).The satiating quality of food decreases throughout the day and therefore, protein intake might increase because protein is more satiating than carbohydrates (de Castro, 2004). However, the breakfast cereals were unfamiliar to a larger percentage of participants and, this could have influenced their ability to discriminate the macronutrient content of the foods. Due to methodological issues with familiarity, the question remains unresolved and further research is needed with revised methodology, before conclusions can be reached about macronutrient valuation across different foods and meal times.

5.1.3 Does an individual's perceived social status influence their macronutrient valuations?

The results in Chapter 4 indicate that perceived social status does influence macronutrient valuations. Not only did participants with lower perceived social status value protein, carbohydrate and fat, on a calorie for calorie basis, more than participants with higher perceived social status, they also valued energy density more. In other words, people with lower perceived social status are more likely to choose energy-dense foods with a higher fat, protein and carbohydrate content. This provides important insight in to the relationship between lower perceived social status and obesity. Perceived social status is not always correlated with socioeconomic status (Adler et al., 2000). Therefore, individuals might appear to have high socioeconomic status but could perceive themselves of a low social standing. The binary forced-choice task could be used to determine whether an individual's perceived social status is making them vulnerable to unhealthy food choices, despite having a high socioeconomic status. Weight loss interventions could then be designed specifically for individuals based on their macronutrient valuations and perceived social status.

5.2 General methodological considerations

A methodological advantage of using a binary forced-choice task to measure macronutrient valuation is the ease-of-completion. The task can be completed on a computer or laptop and is therefore easily transported. Participants select a food using the left and right arrow keys, but the selection processes can be simplified, and food choices could be selected verbally. This might be useful for clinical application, where participants are unable to use the keyboard due to health conditions and age. The food images can also be changed to represent commonly consumed foods in the country of administration. This allows for universal application.

This thesis highlights the importance of familiarity in relation to food choice and macronutrient valuation. This was especially relevant in Chapter 3 with the breakfast cereals. Despite breakfast cereals being a common consumed food at breakfast time (Reeves et al., 2013), a large percentage of participants were unfamiliar with one or more of the breakfast cereals included in the experiment. This could explain why the predicted macronutrient hierarchy protein> carbohydrate> fat was not observed. The familiarity of a food influences the learned associations between the sensory properties and the postingestive feedback of a food (Mccrickerd & Forde, 2016). If an individual has never tasted a food before, then they will not be able to use taste as a signal for the macronutrient content of the food.

Within this thesis, habitual macronutrient intake was not measured. Individual's habitual intake may have influenced their macronutrient valuations or vice versa. For example, individuals who habitual consume a high protein diet could be especially sensitive to small differences in the protein content of foods and may have chosen foods in the tasked based on this (Masic & Yeomans, 2017). However, there is an important distinction between macronutrient intake and macronutrient value. High-protein valuation might promote a greater protein intake, but a greater habitual intake of protein -rich foods does not necessarily translate to higher protein valuation. Habitual food choice is influenced by a multitude of factors such as liking, availability and cost (Blundell & Gillett, 2001; Drewnowski & Specter, 2004). "Macronutrient valuation'" refers to the underlying sensitivity to small differences in protein, carbohydrate and fat and affects all food choices. An individual might have high protein valuation but a low protein intake because of economic restrictions that prevents them from regularly purchasing protein-containing foods.

5.2.1 Limitations

First, the sample recruited for each study was predominantly undergraduate students from the United Kingdom. There was also a slight gender bias, across all of the studies, towards women. This means that the results are not necessarily representative of the population. Additionally, there were concerns in some of the studies (Chapters 3 and 4) regarding the student's socioeconomic backgrounds. The majority of the students were from affluent, well-educated families. Therefore, caution should be taken when generalising the results to broader populations.

As previously mentioned in Chapter 1, macronutrient requirements change with age (Langley-Evans, 2015). However, there was no age restrictions applied to the recruitment process in this thesis. Initially, this was to ensure that the recruitment criteria were not too stringent. The large age range could have contributed to the large variation between participants in their macronutrient valuations. In future studies, to explore how macronutrient valuations differ with age, participants from specific age ranges should be recruited.

It is important to note that the timing of the study may have influenced the participant's food choices, especially in Chapter 3 where two different meal times were included. Across all of the studies, in this thesis, experiment time slots were scheduled between 14:00 and 18:00. Therefore, participants were selecting breakfast cereals at a time of day that is not typically associated with breakfast. It could have been beneficial to have two separate test sessions, the first in the morning and the second at midday. Participants could then make their food choices at the time of day that the food was typically consumed.

Additionally, if the study was to be repeated in the future, a measure of participant awareness should be included. It was not obvious as to whether the participants knew the aim of the experiment and if so, whether they were selecting food based on their suspected aims. Therefore, including a measure of experimental awareness would be useful to further understand participant's food choice motivations. For example adding a question such as "What do you think this study was investigated?" This could also give a further insight in to whether the amount of value an individual placed on protein, carbohydrate and fat is a conscious or unconscious decision.

5.3 Future directions

One of the aims of this thesis was to develop a novel tool to measure the relative value that individuals placed on protein, carbohydrate and fat, on a calorie for calorie basis. This thesis demonstrates that the binary forced-choice task is an effective tool to quantify individual's macronutrient valuations and has excellent test-retest reliability. The task is versatile and could be paired with additional measures to further investigate factors that influence macronutrient valuations.

For example, a measure of physical activity could be incorporated to explore whether macronutrient valuations change after intense exercise. Previous research has failed to see an increase in carbohydrate intake after participants performed physical activity (Lluch, King, & Blundell, 1998). Although intake does not increase, preferences for carbohydrate (sucralose) solutions increase after cycling (Horio, 2004). After performing physical activity, the muscles glycogen stores are depleted (Kuipers, Keizer, Brouns, & Saris, 1987). Carbohydrate valuation might increase after physical exercise because individuals are looking to replenish their glycogen stores. In order to regulate macronutrient intake, individuals are more likely to select a carbohydrate-rich food, after exercise to increase glucose in the blood. This would indicate that the sensitivity to differences in carbohydrate compositions might be heightened after physical activity to maintain carbohydrate balance.

The binary forced-choice task is easy to administer and could be used for clinical purposes. As previously mentioned, macronutrient intake requirements change over the lifespan, especially protein intake. As humans age, muscle-mass deteriorates and there is an increased risk of sarcopenia (Beasley et al., 2013). Sarcopenia can negatively affect the ability to perform daily tasks and can result in accidents and bone fractures (Shlisky et al., 2017). Appetite can also reduce with age and therefore many elderly people do not consume their recommended amount of protein. In order to reduce the risk of muscle-loss and injury, it is essential to consume an adequate amount of good quality protein with every meal (Paddon-Jones & Rasmussen, 2009). Individuals with low protein valuation might be at risk for inadequate protein intake because they find it harder to identify the protein content of foods. Therefore, the binary forced-choice task could help identify individuals who are vulnerable to sarcopenia. These individuals can then receive directed dietary advice that can identify and encourage eating protein-rich foods.

The binary forced-choice task could be used to determine whether humans can discriminate the carbohydrate quality of different types of carbohydrate sources, particularly simple sugars and complex starch foods. Individuals might then show differential carbohydrate valuations based on their ability to discriminate the carbohydrate quality of foods. This could be particularly useful in clinical settings to help identify an individual's risk of developing type 2 diabetes, alongside additional measures. Type 2 diabetes is the most common type of diabetes and arises because of an insulin deficiency. The body cannot produce enough insulin or the body's cells do not react to insulin and this can lead to a build of glucose in the blood (Smushkin & Vella, 2010). There has been an increased interest in the relationship between the quality of carbohydrates in the diet and diabetes. The glycemic index of a food influences the blood glucose levels and thus the amount of insulin released (Wolever et al., 1991). Evidence indicates that the risk of developing type 2 diabetes can increase, if a large quantity of foods, with a high-glycemic index is regularly consumed. Whereas, a high intake of cereal and fruit fibre (low-glycemic index) can decrease the risk of developing type 2 diabetes (Schulze et al., 2004). Individual's with a high carbohydrate valuation for simple sugar foods might have a greater risk of developing type 2 diabetes. These individuals will often have a higher sensitivity to the carbohydrate content of foods and are more likely to reject a food with a lower carbohydrate content. This highlights the importance of carbohydrate quality as well as quantity. Individuals who demonstrate a higher value for carbohydrate-rich foods with a high-glycemic index could receive valuable dietary advice about carbohydrate quality and the recommended quantity that could reduce their risk of developing type 2 diabetes.

Recently, there have been growing concerns about the sustainability of our diets. There are considerable ecological and environmental issues associated with meat production (Dauvergne, 2008) and there have been several attempts at trying to reduce the populations meat consumption (Tobler, Visschers, & Siegrist, 2011). However, there are many people, considered "meat-eaters" who believe that meat is a staple and essential part of their diet (Dagevos & Voordouw, 2013). In order to meet protein requirements, protein intake does not need to be consumed solely from meat-products. Eggs and cheese also contain a high percentage of protein as energy. Vegetarian diets do not include meat or fish and vegan diets are absent of all animal products (Bradbury, Tong, & Key, 2017). It can be challenging for individuals who consume vegan diets to meet their recommended protein intake, due to the absence of animal products (Le & Sabaté, 2014).

This raises an interesting question about whether protein would be prioritized more by individuals who consumed vegetarian and vegan diets or by meat-eaters. Plant-based protein sources (e.g. soy and tofu) could be added alongside the animal-based protein sources (e.g. chicken and beef) in the binary forced-choice task. Vegetarians and vegans might have a high protein valuation and be particularly sensitive to small differences in protein because the variety of protein sources available to them is restricted. Although, meat-eaters could be also be sensitive to small differences in protein because they regularly consume high-protein foods and might have 'strengthened' their ability to detect the protein content of a food through experience. Research in to this area could provide important insights in to people's attitudes towards plant-based protein sources and could potentially help develop strategies to reduce meat consumption and increase sustainable diets.

5.4 Closing remarks

This thesis includes a collection of experiments that, for the first time address the idea of macronutrient valuations. The binary forced-choice task was developed to quantify and identify individual's sensitivity to differences in macronutrient composition rather than overall macronutrient intake. The results suggest that all calories are not the same and that individual differences moderate macronutrient valuations, however there are still some questions to be answered. The methodology developed for this thesis has strong test-retest reliability and provides the basis and justification for future research.

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